



*Molecules to Medicine*  
*Molecular Biology Sub-Block*

# Tools of Molecular Biology II

Matthew Taliaferro, PhD

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[matthew.taliaferro@cuanschutz.edu](mailto:matthew.taliaferro@cuanschutz.edu)

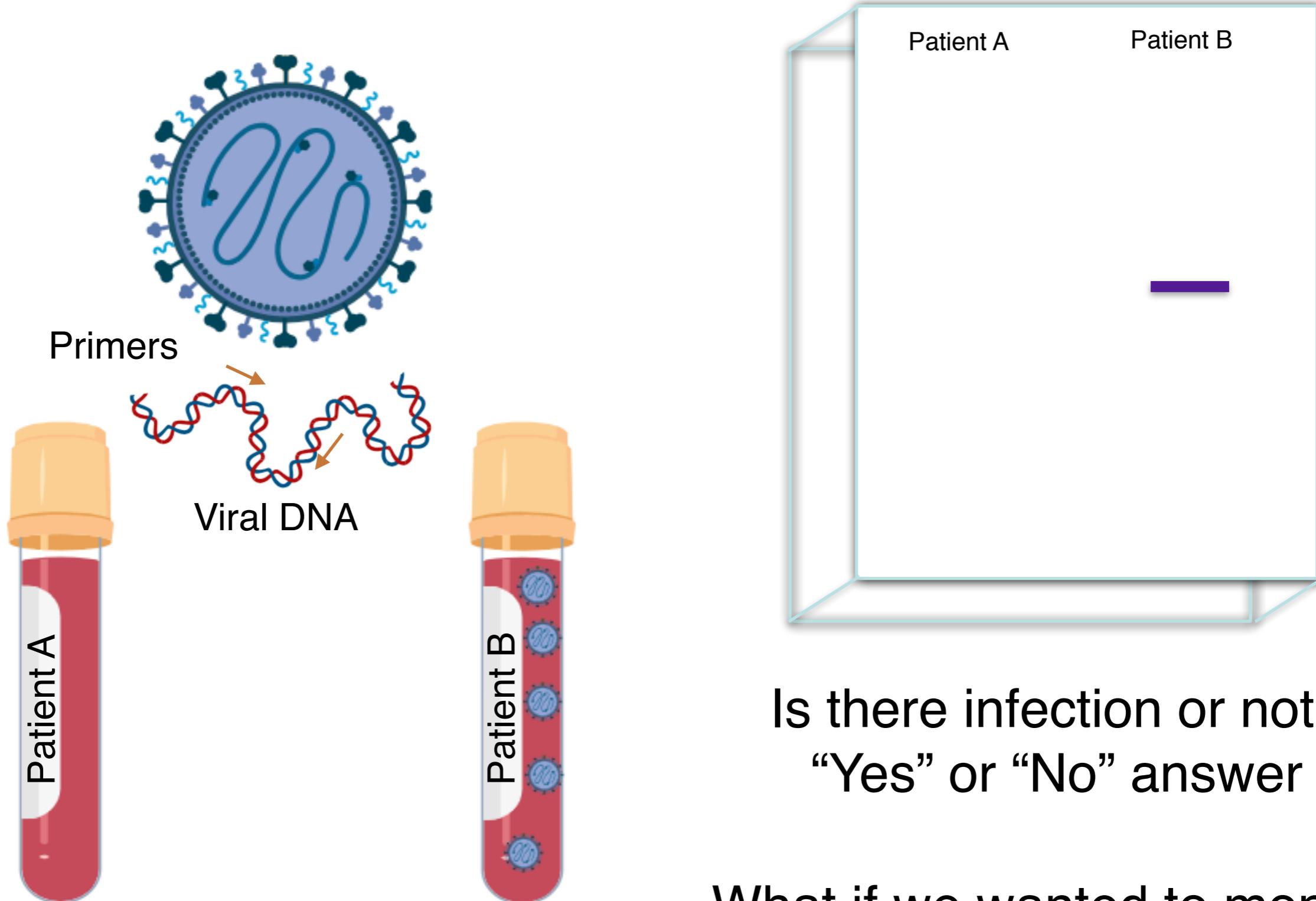
# Objectives

1. Describe the use of microarrays for measuring levels of mRNA gene expression and their implications for diagnosis and treatment.
2. Describe the process of producing recombinant proteins and provide examples of their utility in medicine.
3. Describe standard approaches used in recombinant molecular biology approaches to: a) copy a DNA sequence into a DNA sequence, b) copy an RNA sequence into a DNA sequence, and c) join DNA fragments.
4. Describe different types of cloning vectors and their general applications.
5. Describe the principles behind real time PCR and its application to the diagnosis or monitoring of infection.
6. Describe the use of antibody-based protein quantification techniques and their implications for diagnosis and treatment.

# Outline

- **Multiplexed qPCR and microarray analysis**
  - Quantifying the expression of many genes at once
- **Recombinant protein production**
  - Industrial scale production of medically relevant polypeptides
- **Antibody-based protein quantification**
  - Immunoblotting, ELISA, and their applications in diagnostic techniques

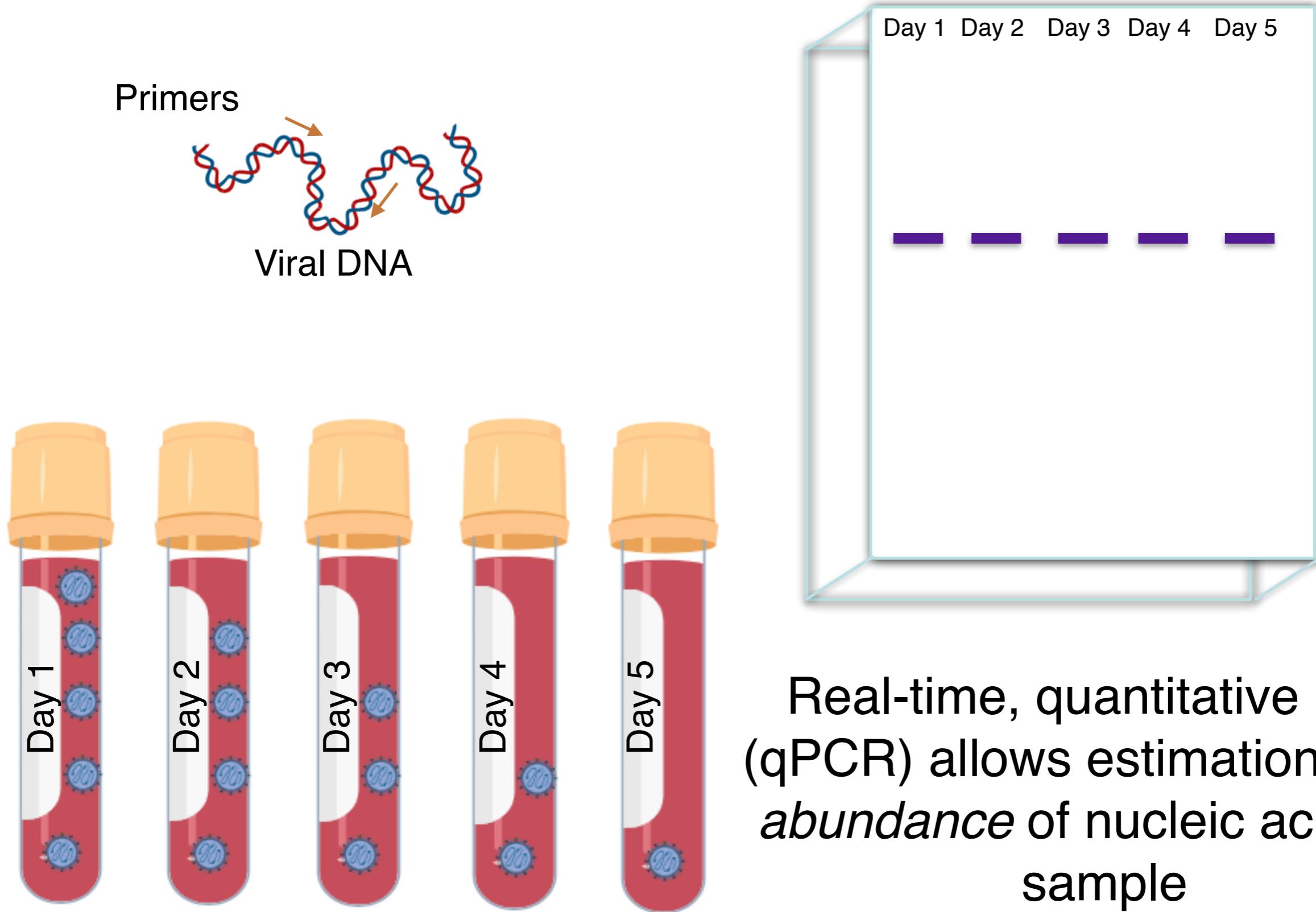
# Assaying infection with PCR



Is there infection or not?  
“Yes” or “No” answer

What if we wanted to monitor  
extent of infection over time?

# Assaying infection with PCR

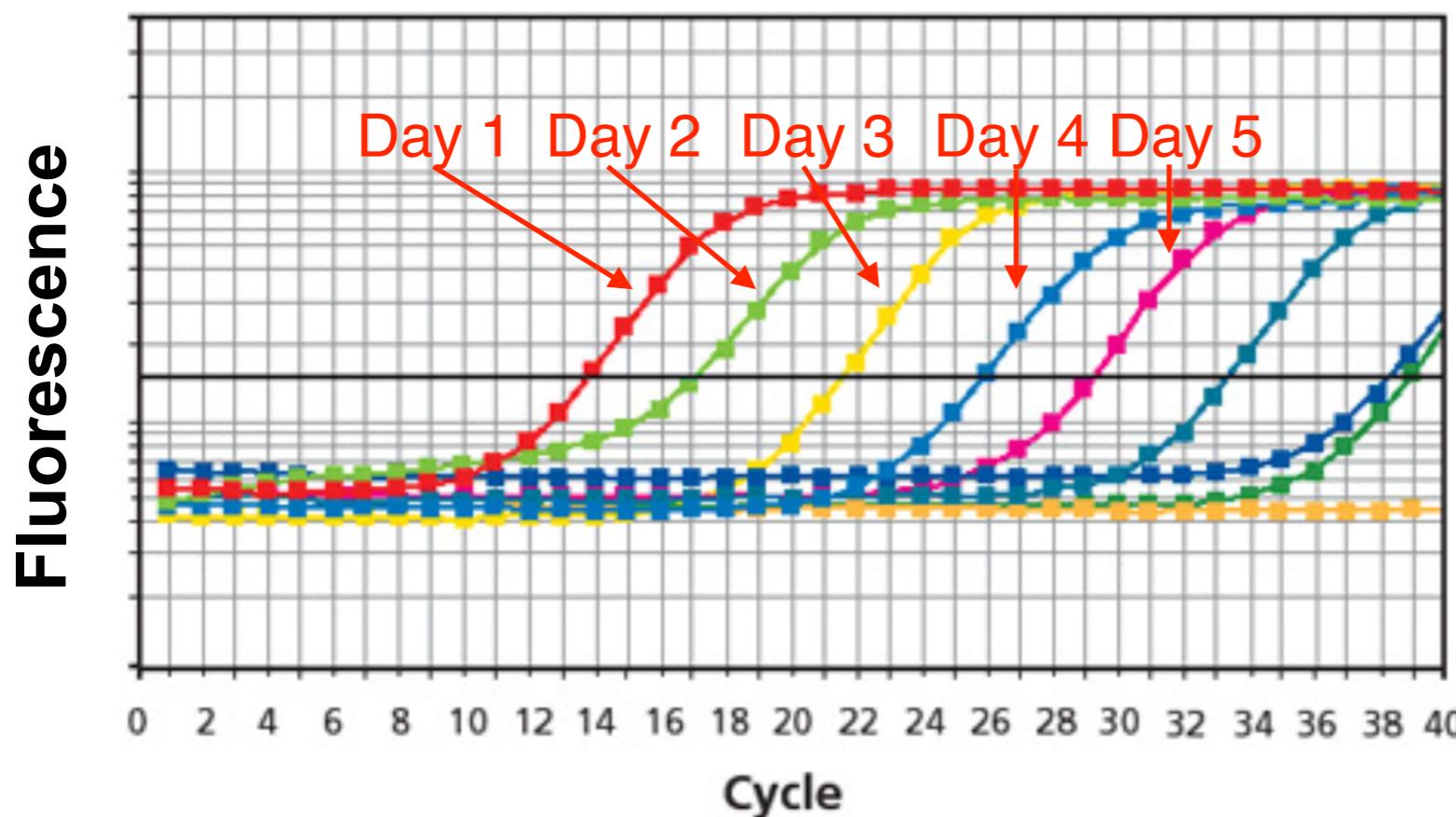


# Real-time, quantitative PCR (“qPCR”)



1. Dye in solution emits low fluorescence

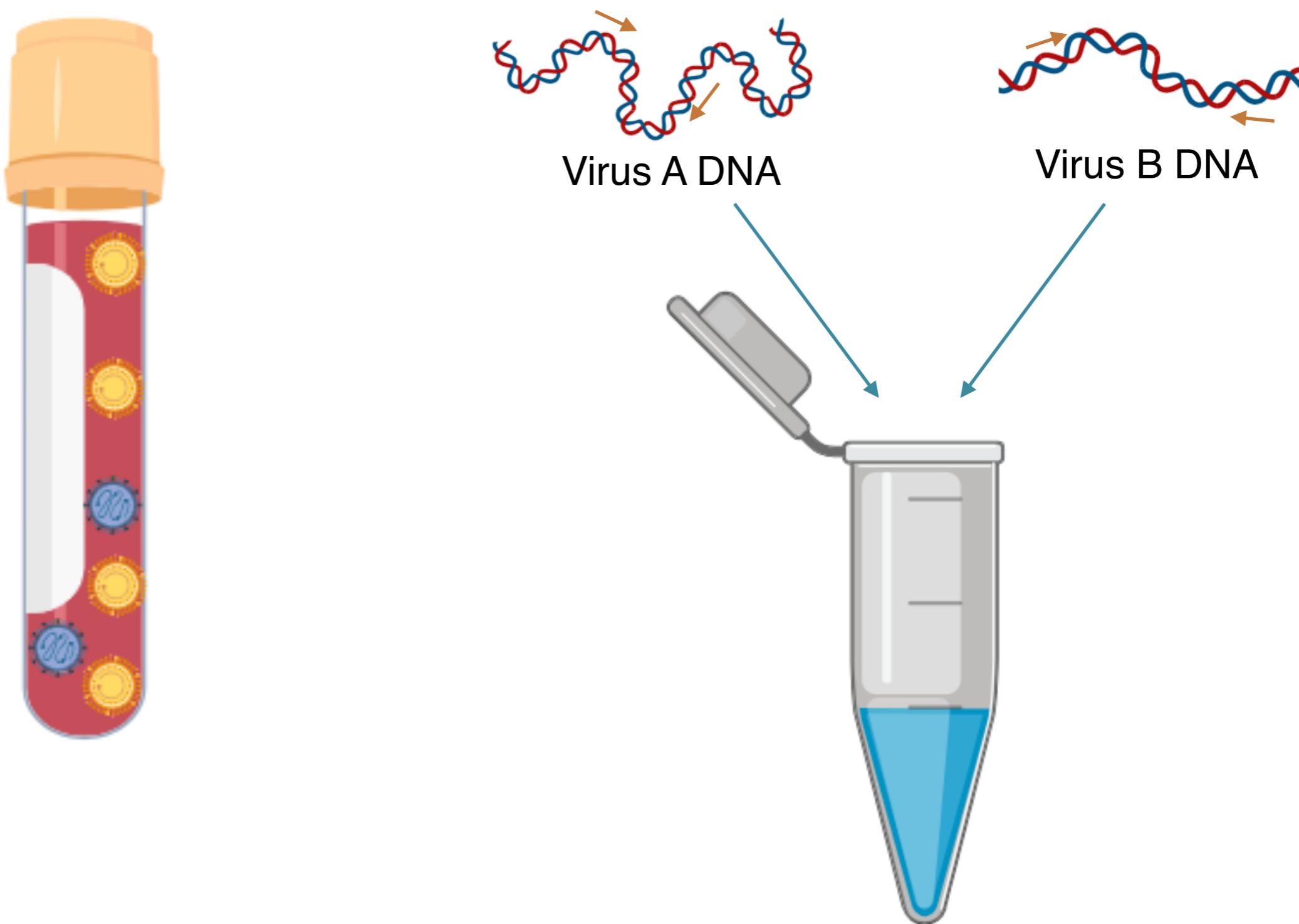
2. Emission of the fluorescence by binding



Direct readout,  
so no analysis  
by gels

# Multiplexing qPCR

What about quantifying two viruses at once?



# QUIZ

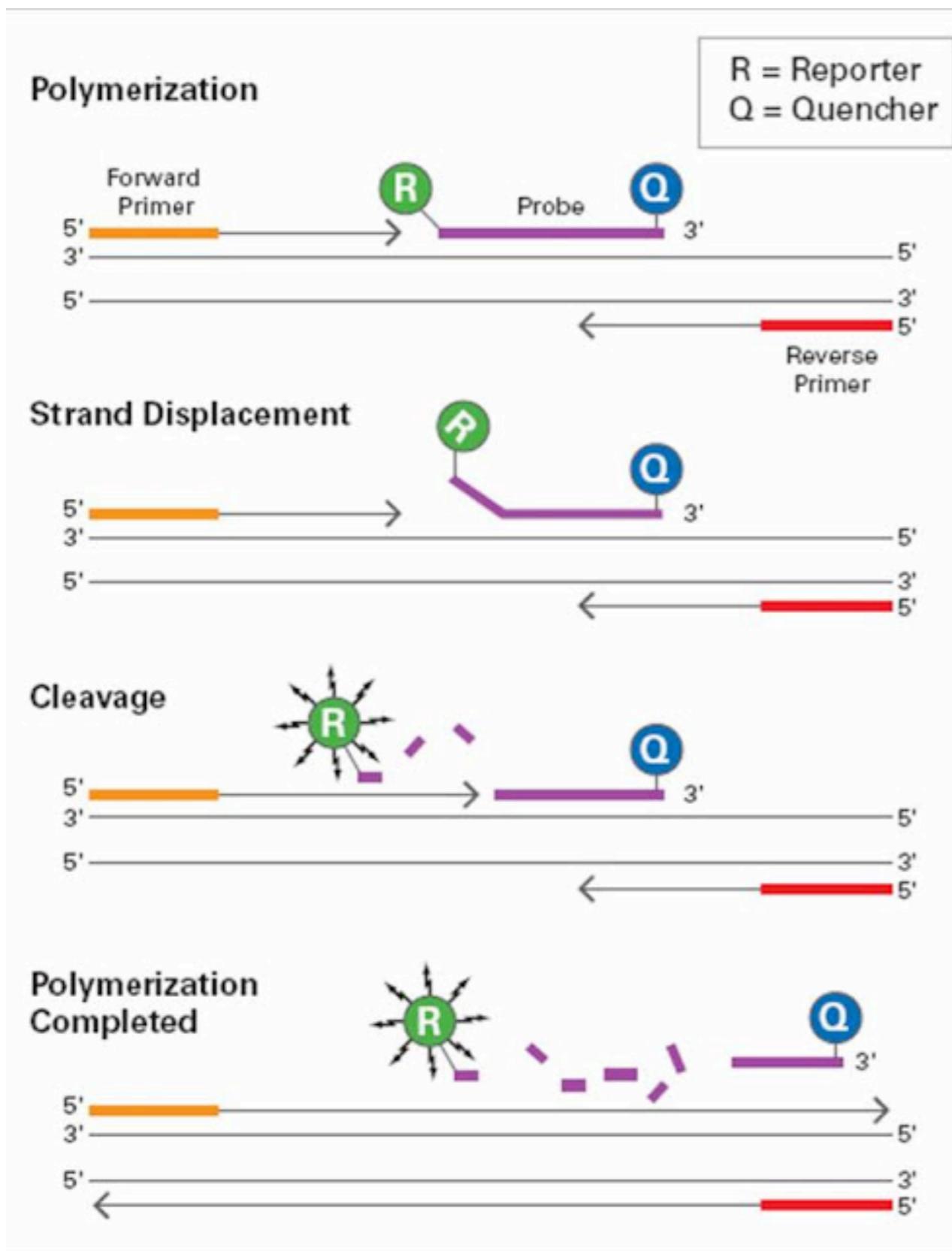
You have a patient that you believe is infected with two distinct viruses. Why can you not monitor the extent of infection of both viruses independently using a *single* (not multiplexed) qPCR assay?

# QUIZ

You have a patient that you believe is infected with two distinct viruses. Why can you not monitor the extent of infection of both viruses independently using a *single* (not multiplexed) qPCR assay?

- A. You usually only get enough DNA from a blood sample to assay for one virus.
- B. It is impossible to perform two independent PCR reactions in the same tube at the same time.
- C. The amount of DNA primer you would have to put in to amplify two viruses would inhibit the reaction.
- D. The dye used to detect DNA in qPCR binds the amplification products of both viruses equally, preventing the determination of which virus it came from.

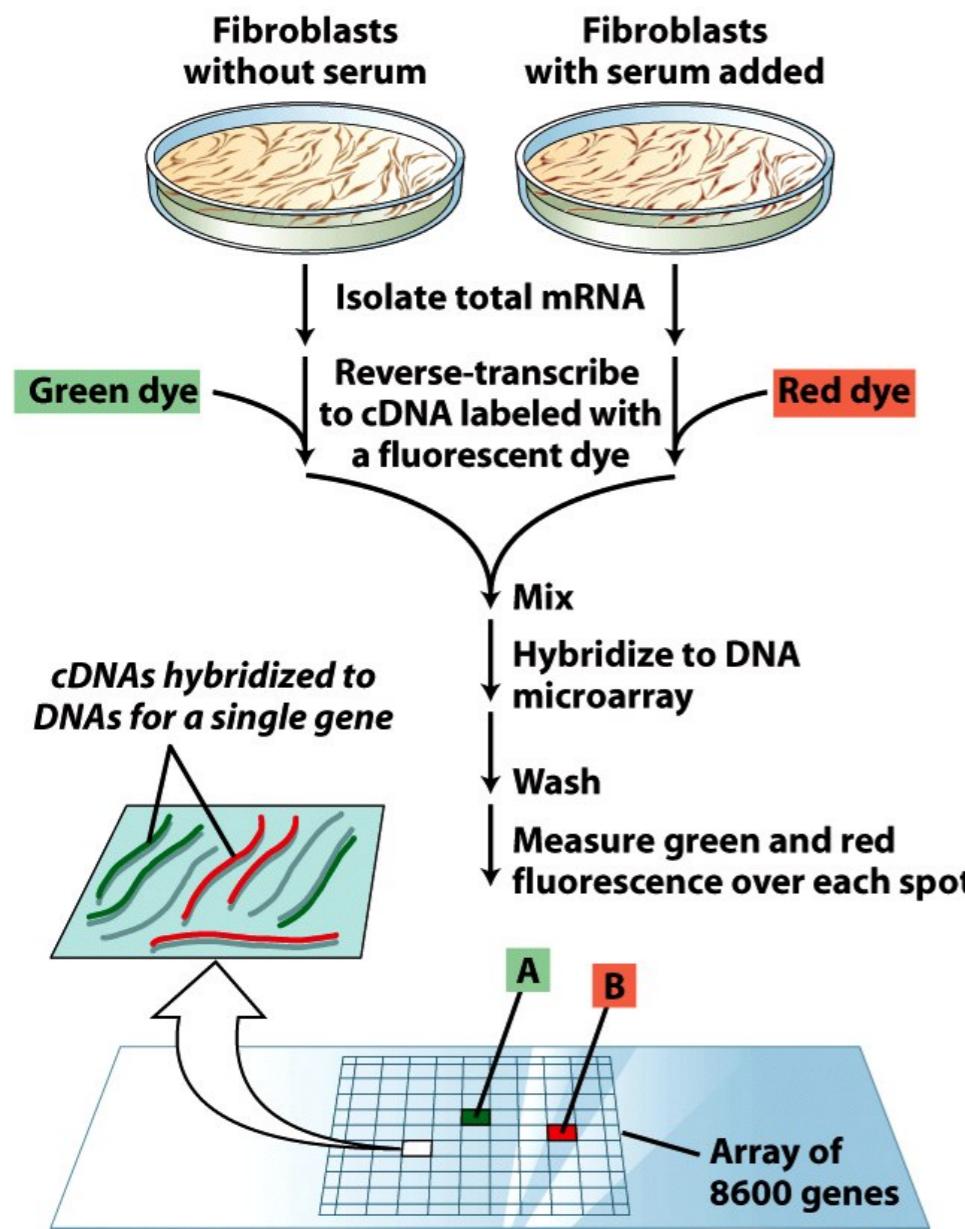
# Multiplexing qPCR



Quantify fluorescence from each reporter after each cycle.

Each species of interest therefore has its own amplification curve.

# Microarray Analysis



- A** If a spot is green, expression of that gene decreases in cells after serum addition
- B** If a spot is red, expression of that gene increases in cells after serum addition

- Developed to simultaneously analyze the expression patterns of thousands of genes
- Microarray chips contain hybridization probes complementary to fluorescently-labeled nucleic acids from source of interest
- Comparisons in global gene expression can be made between different conditions
  - tumor vs. normal, before and after growth factor addition, etc.

## Microarray result

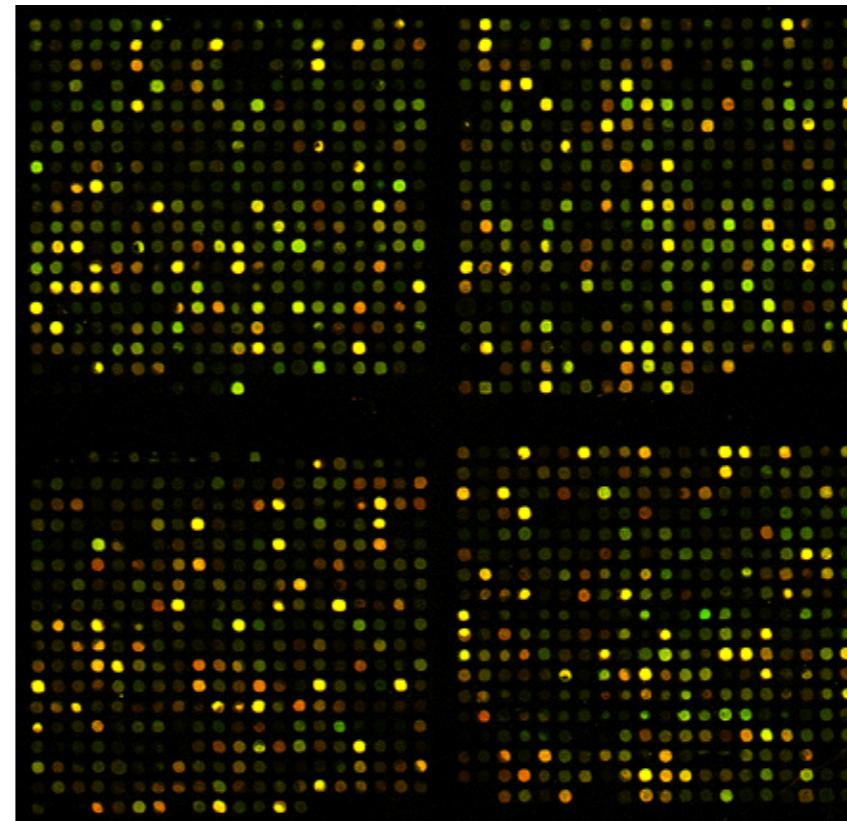


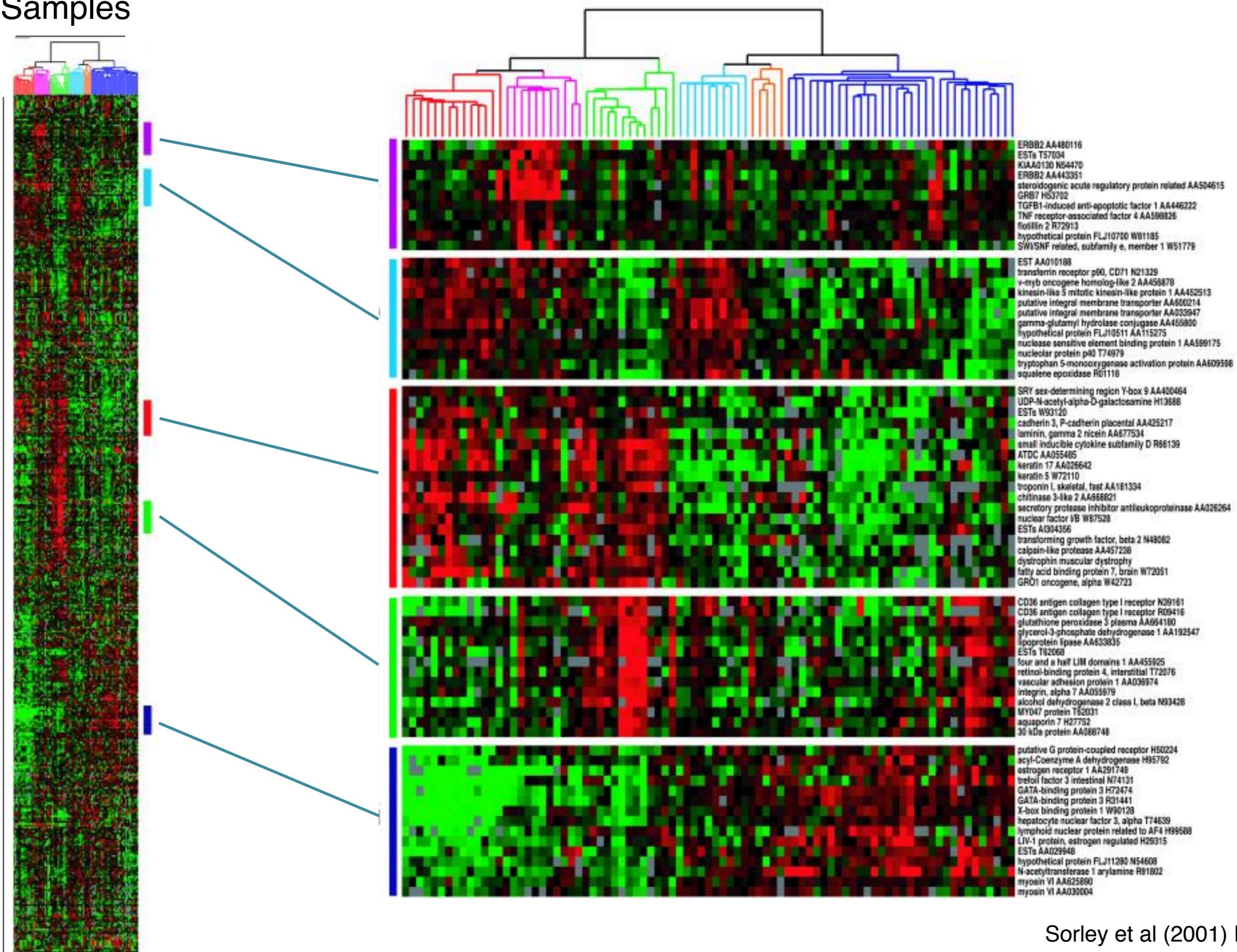
Table of fluorescence intensities (i.e. mRNA abundances) for each feature on the array

Figure 5-29a  
*Molecular Cell Biology, Sixth Edition*  
© 2008 W.H. Freeman and Company

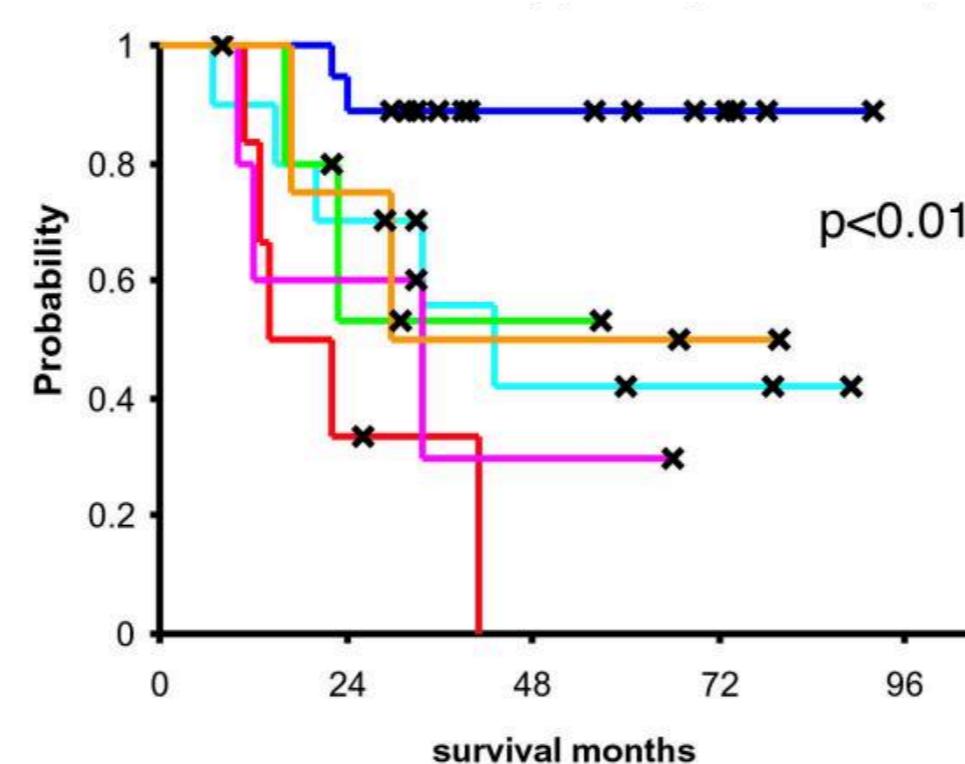
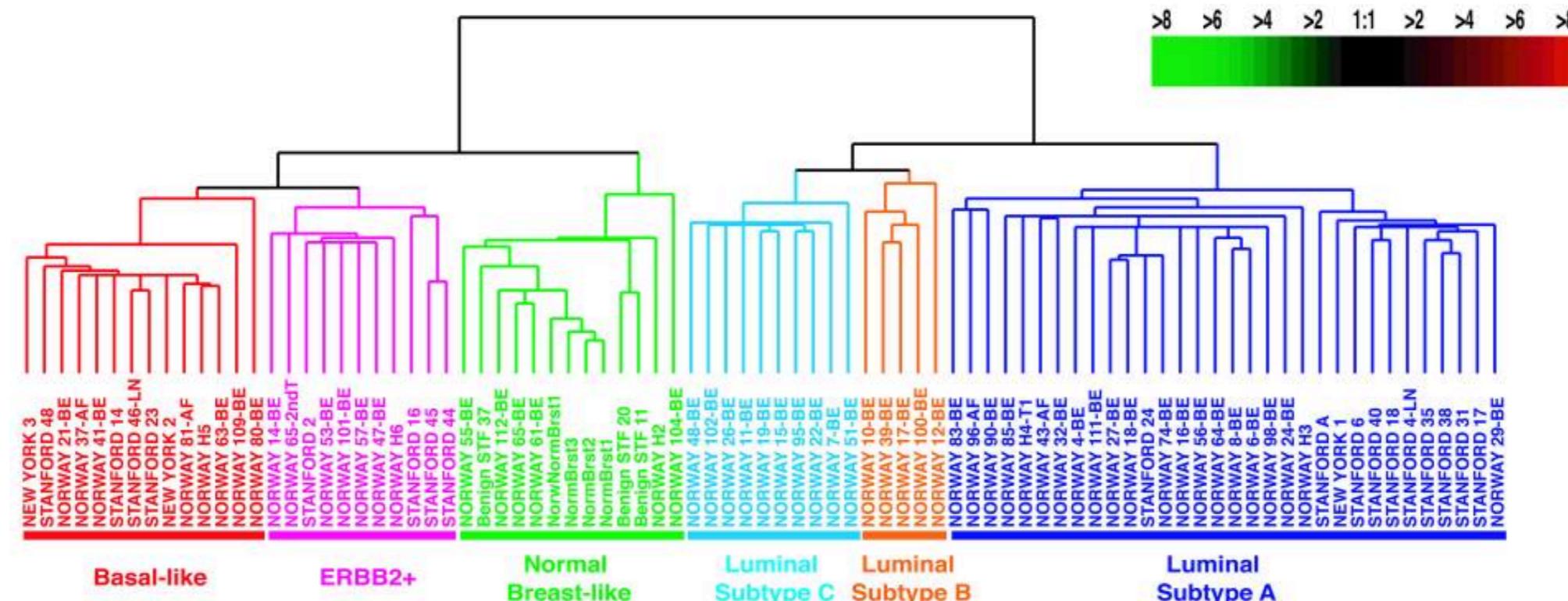
# Microarrays for classifying cancer subtypes

Samples

Genes

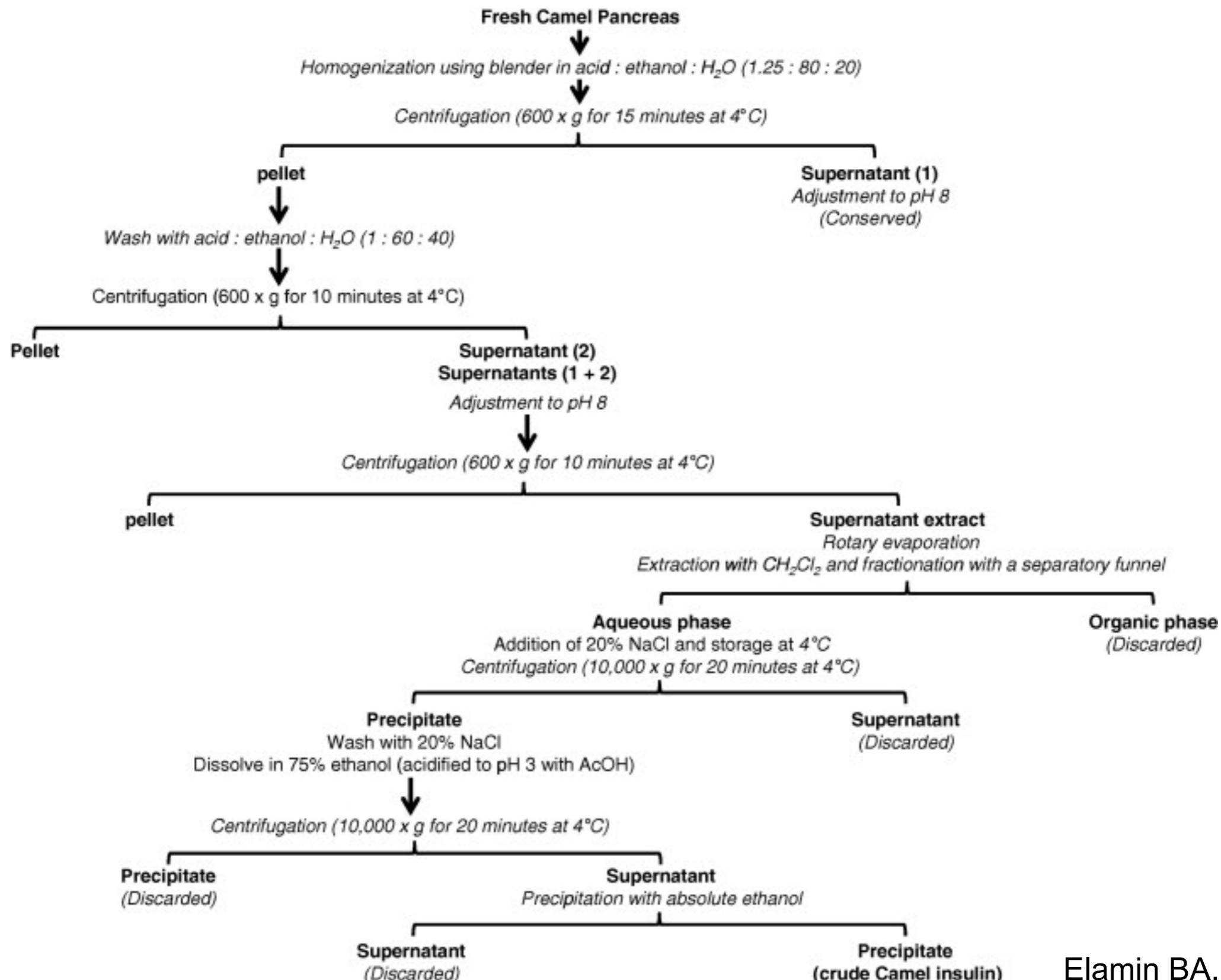


# Microarrays for classifying cancer subtypes

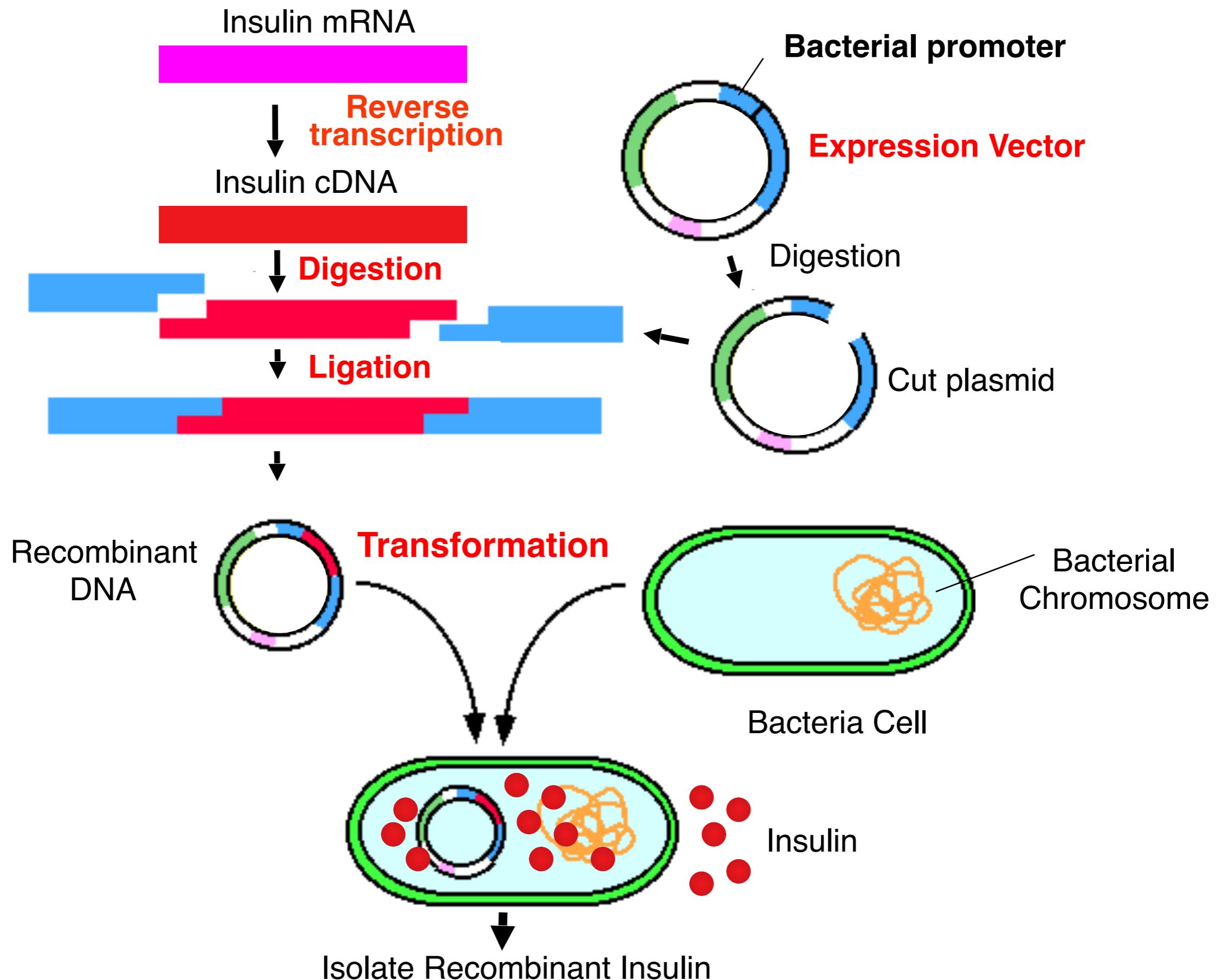


X Censored, — Lum A, — Lum C, — NorB-like,  
— Basal, — ERBB2+, — Lum B

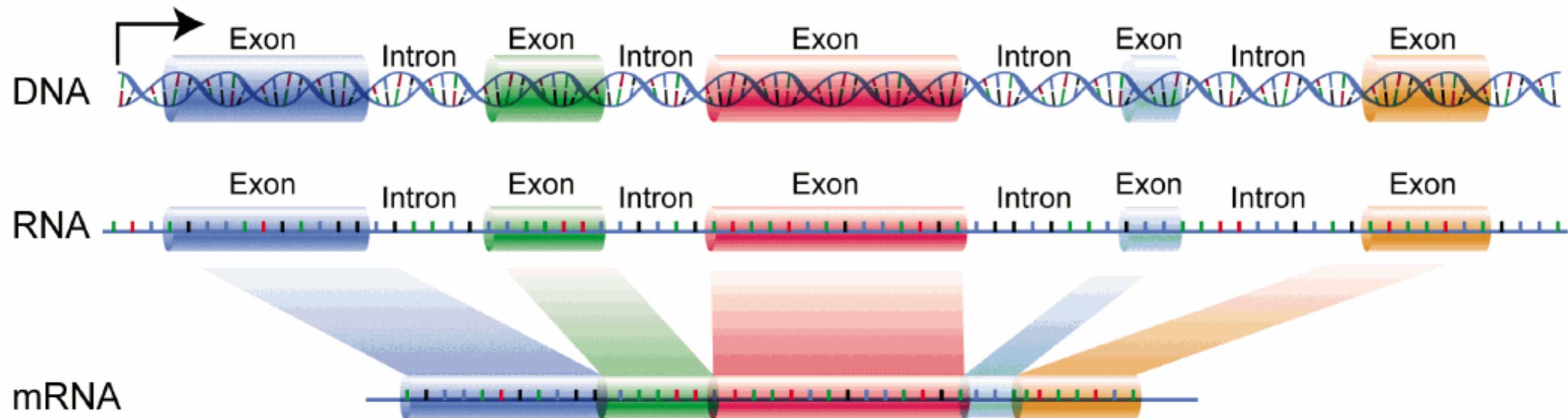
# Purification and functional characterization of pancreatic insulin from camel



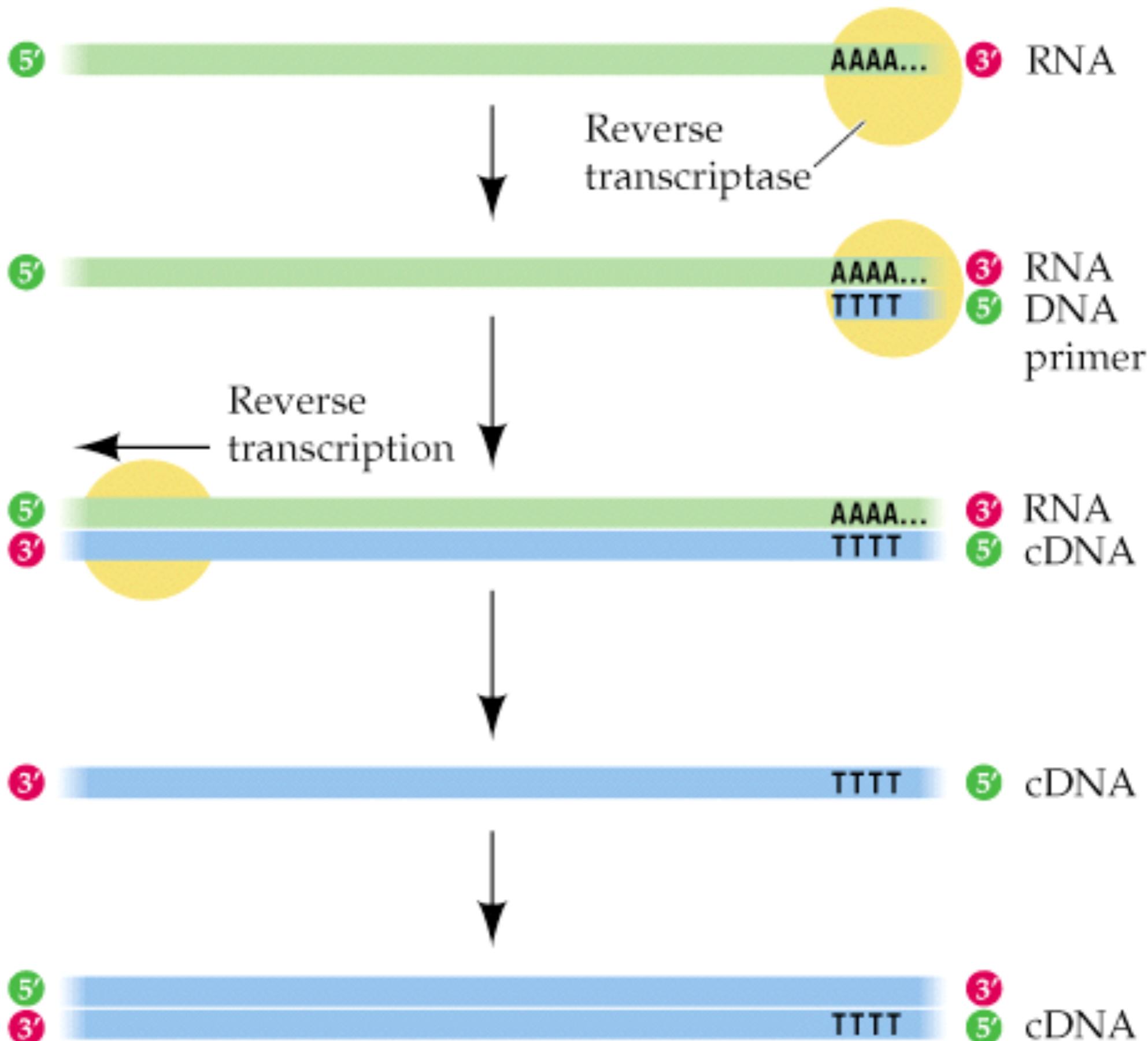
# Expression of recombinant human insulin in *E. coli*



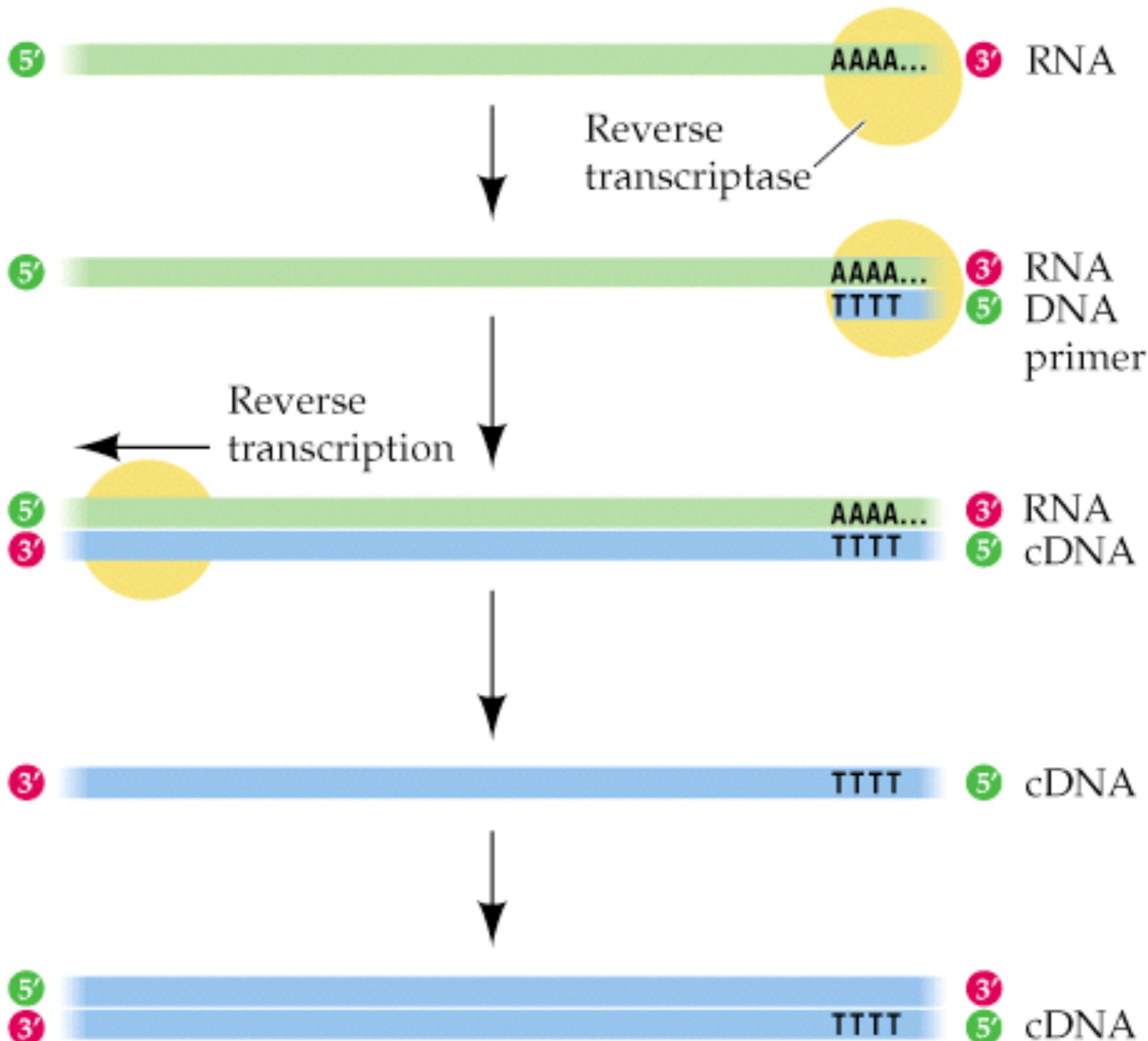
# Why do we need to reverse transcribe? Why can't we just use PCR to amplify the insulin gene straight from the genome?



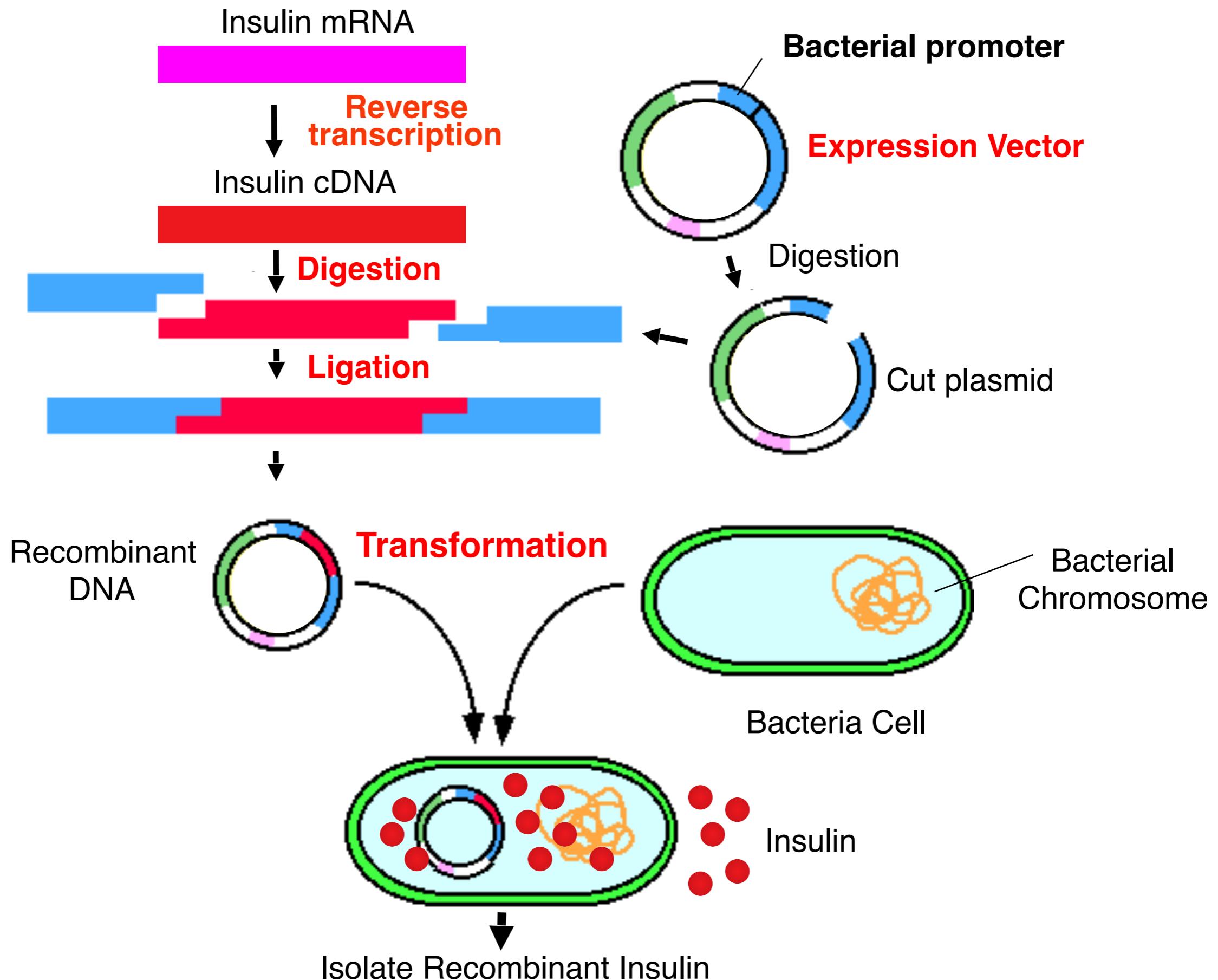
# Reverse transcriptase copies RNA to DNA



# Reverse transcriptase copies RNA to DNA

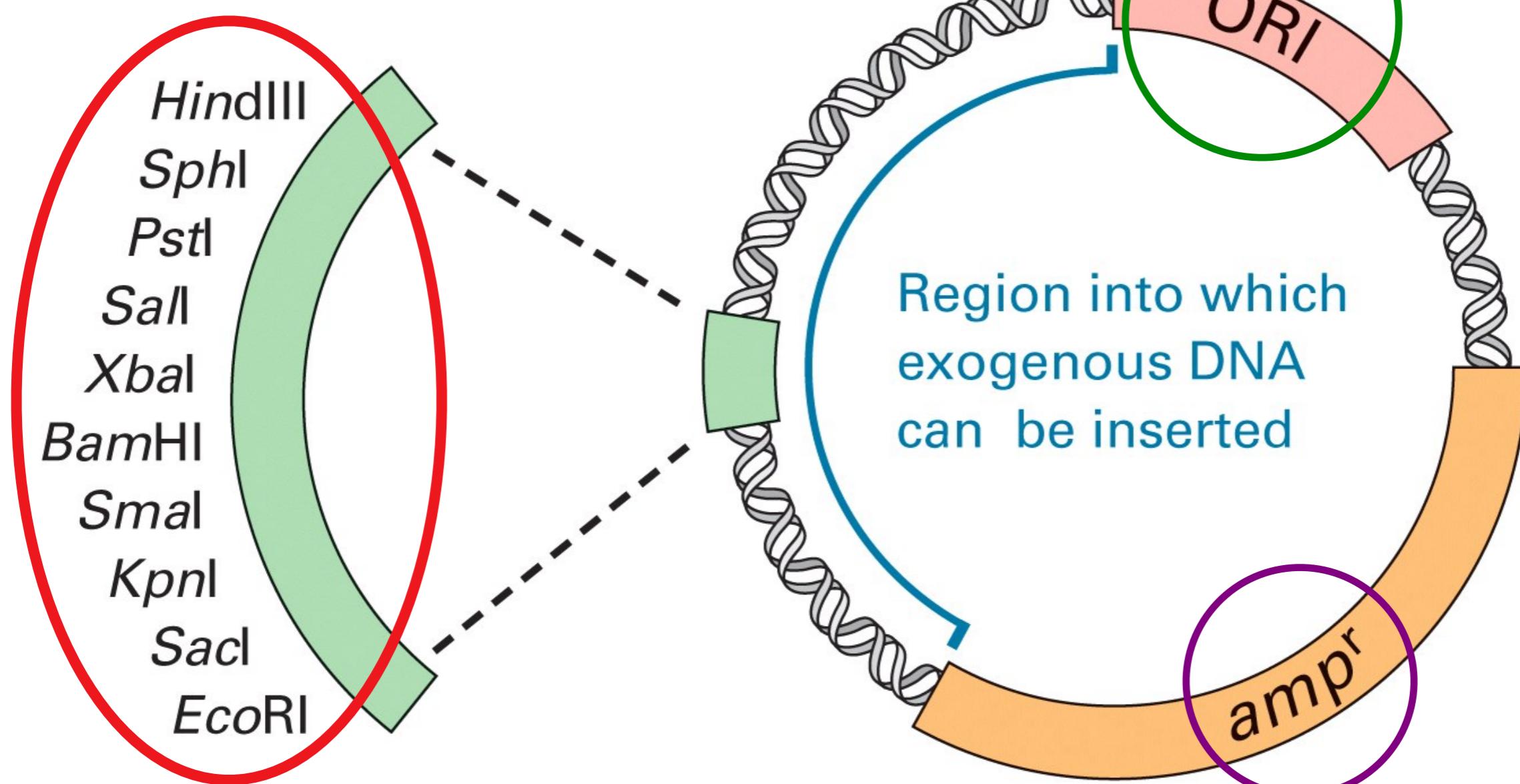


# Expression of recombinant human insulin in *E. coli*



# Key features of cloning vectors

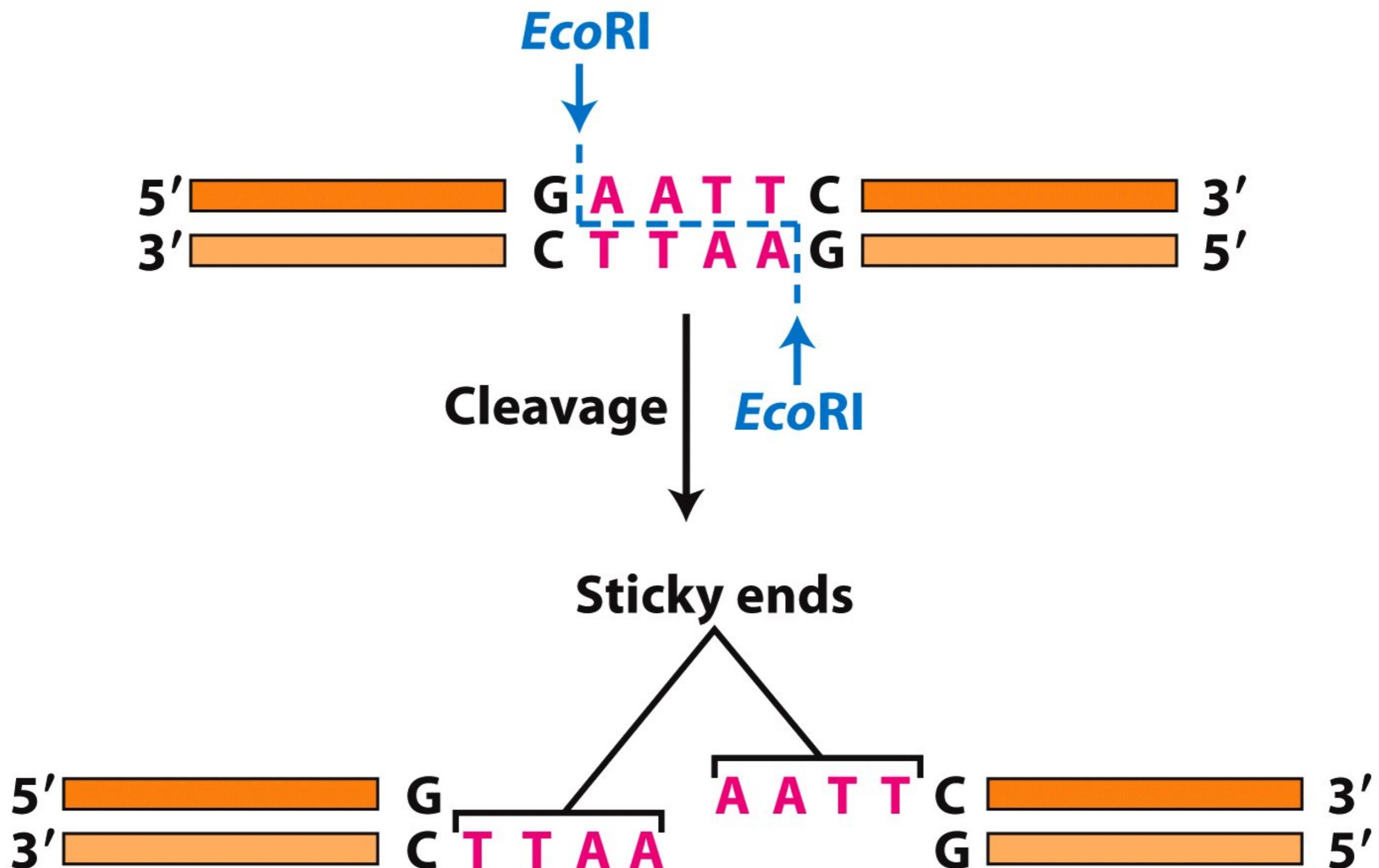
## 2. Origin of replication



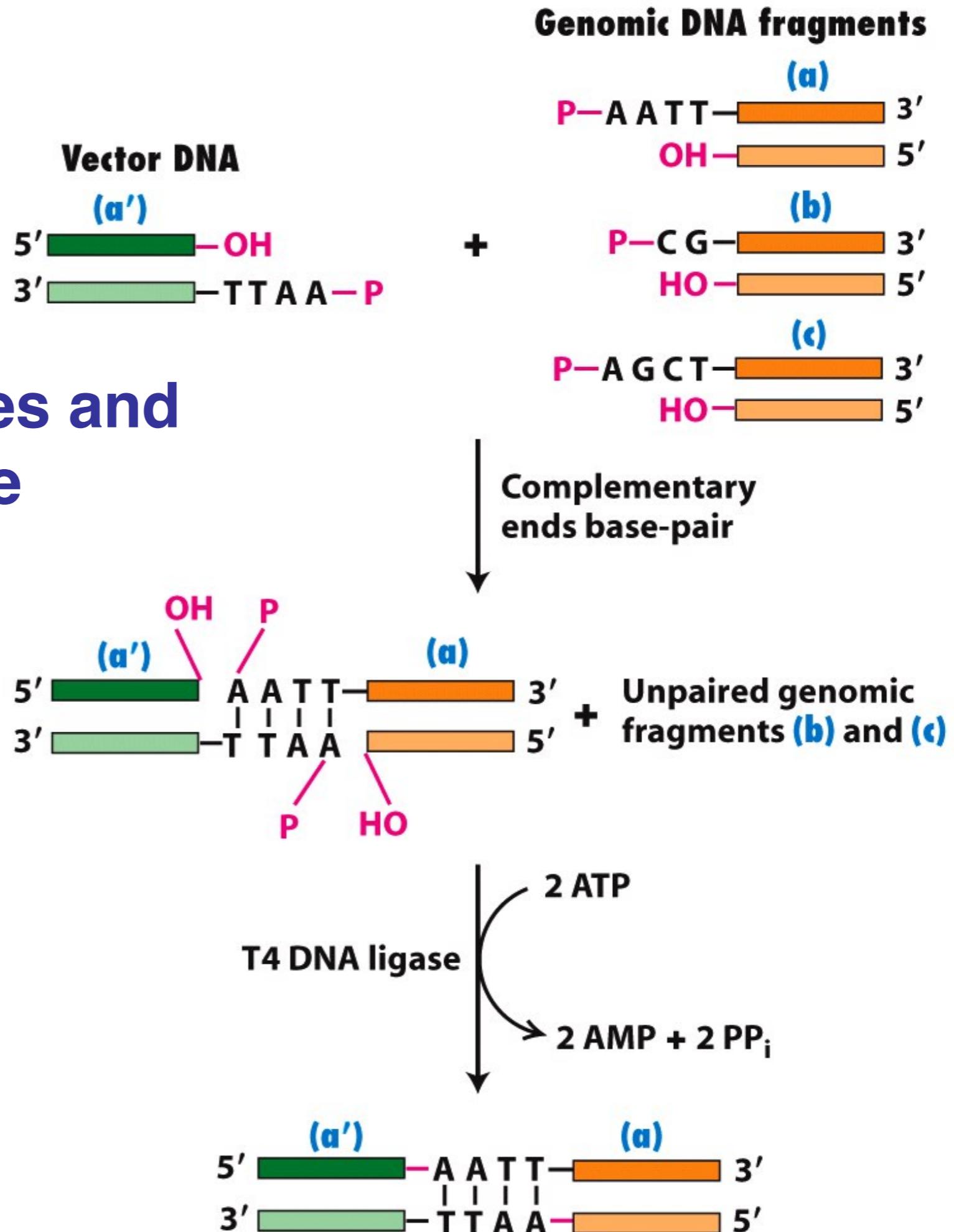
## 1. Restriction sites

## 3. Selectable marker

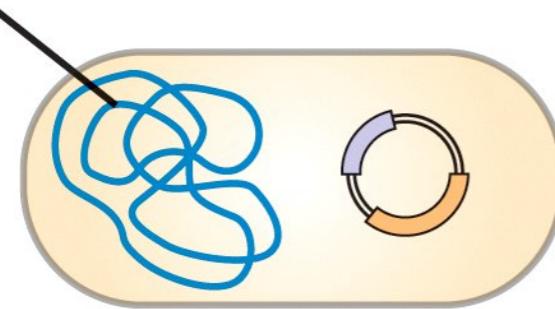
# Restriction enzymes recognize palindromic DNA sequences (usually)



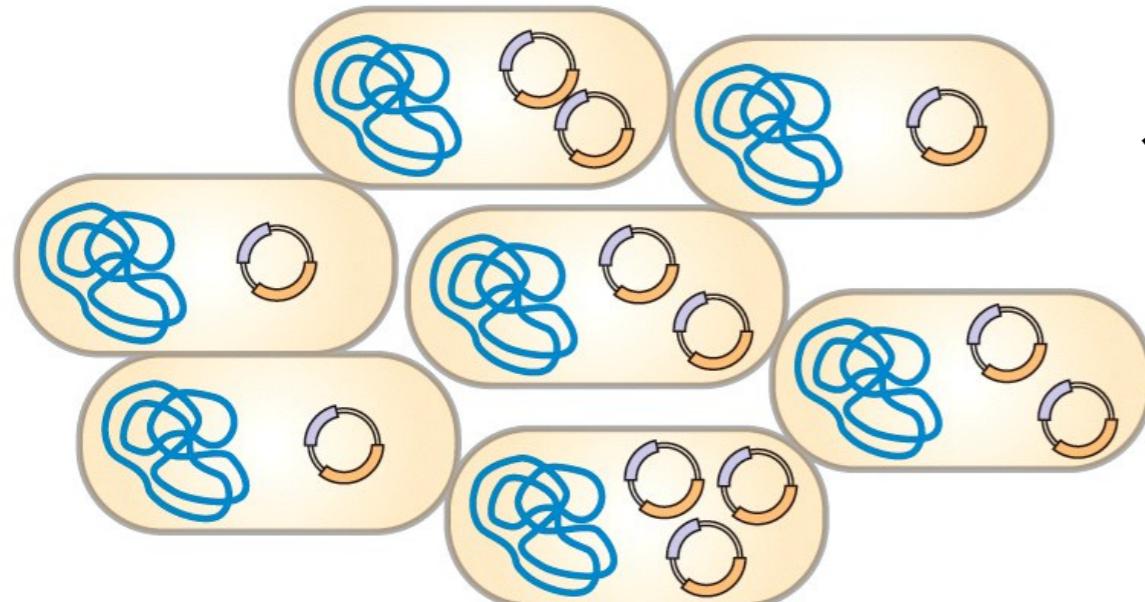
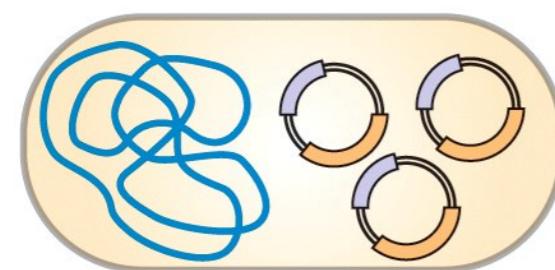
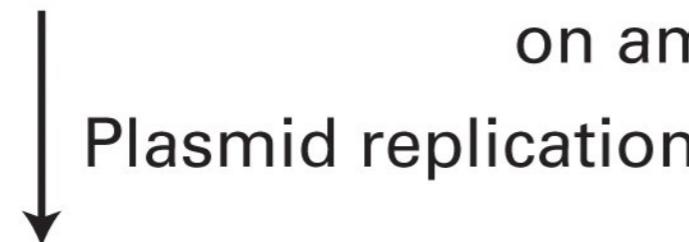
## Use of restriction enzymes and DNA ligase to make recombinant DNA



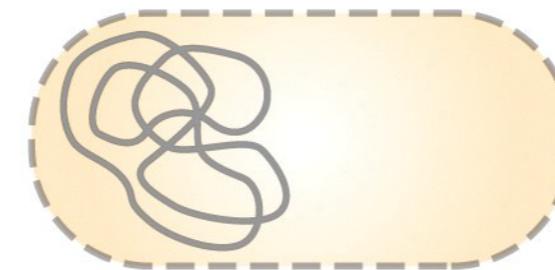
*E. coli*  
chromosome



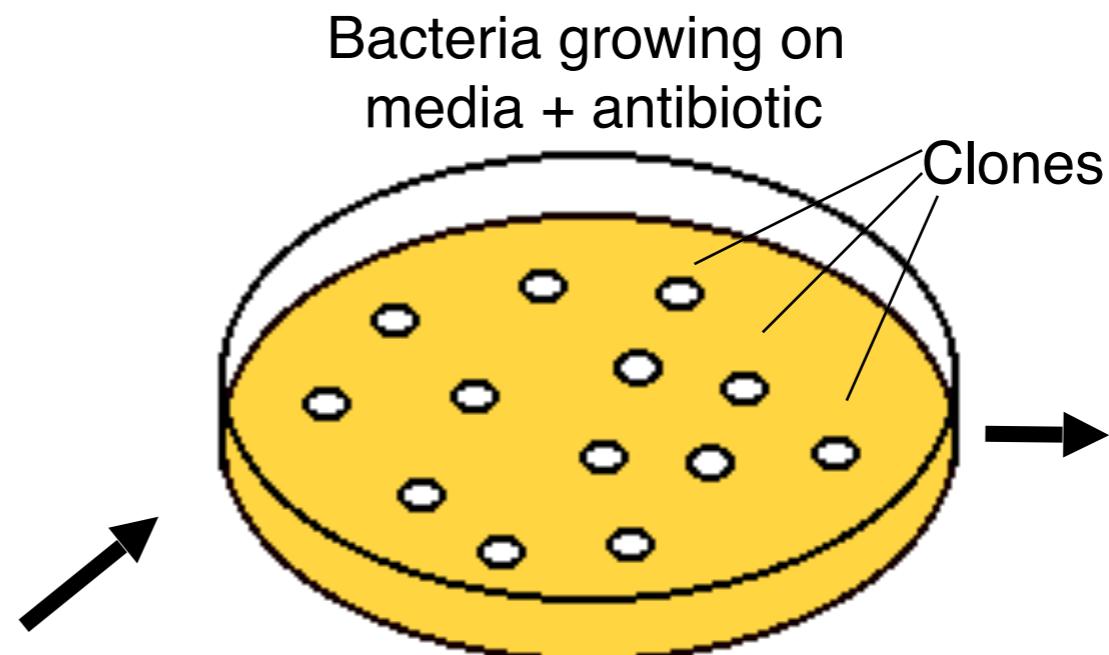
Transformed cell  
survives



# Transforming a plasmid into *E. coli* for cloning purposes



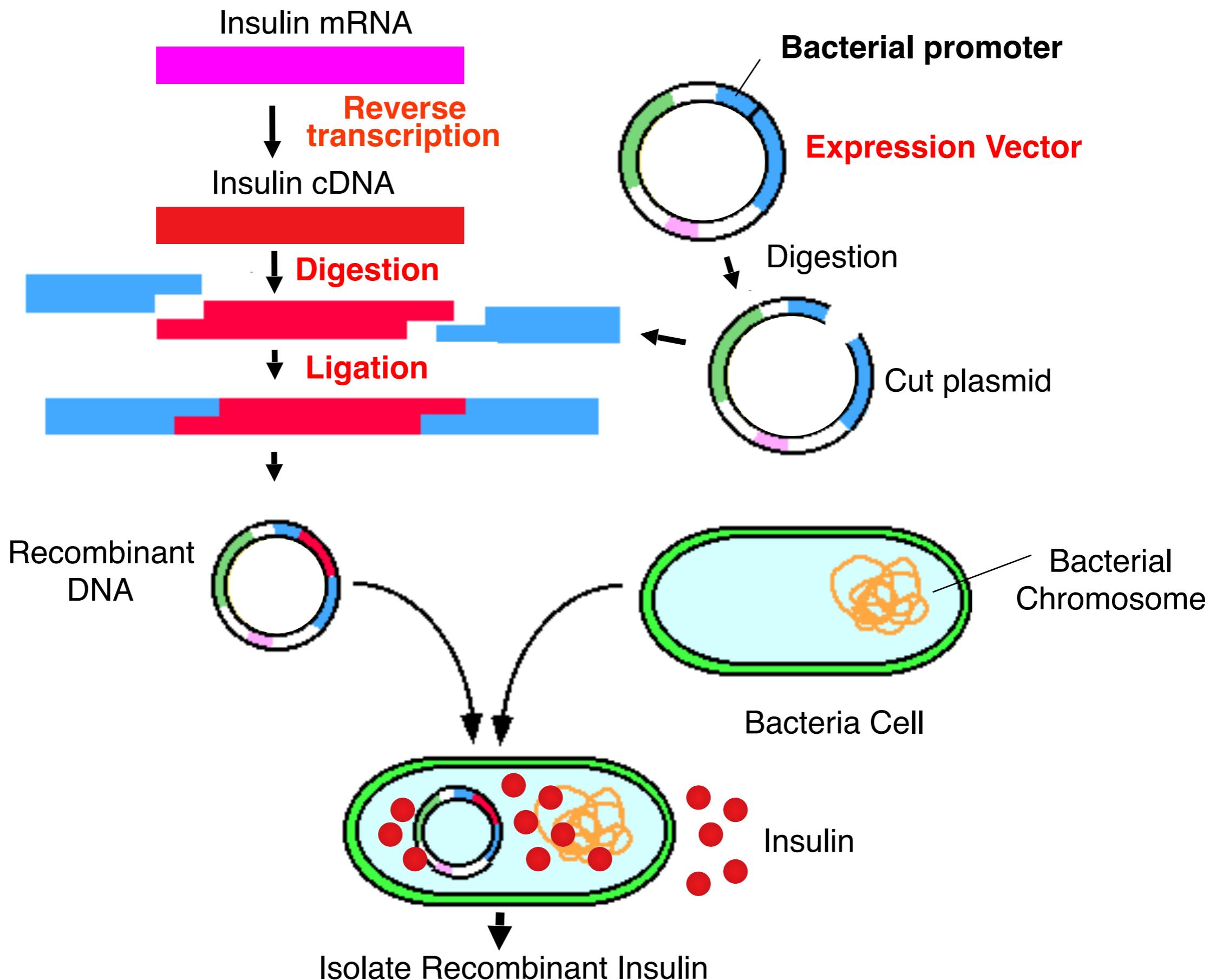
Cells that do not  
take up plasmid die  
on ampicillin plates



Colony of cells, each  
containing copies of the  
same recombinant plasmid

Screen  
recombinant  
clones to find  
correct ones

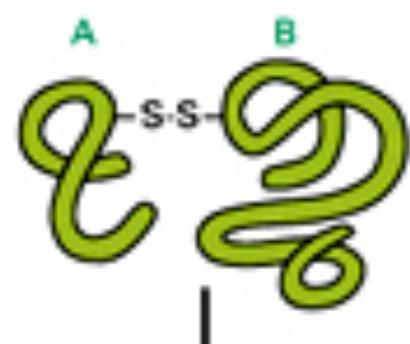
# Expression of recombinant human insulin in *E. coli*



# Some Recombinant DNA Products in Medicine

Product category	Examples/uses
Anticoagulants	Tissue plasminogen activator (TPA) activates plasmin, an enzyme involved in dissolving clots; effective in treating heart attack victims.
Blood factors	Factor VIII promotes clotting and is deficient in hemophiliacs; use of factor VIII produced by recombinant DNA technology eliminates infection risks associated with blood transfusions.
Colony stimulating factors	Immune system growth factors that stimulate leukocyte production; used to treat immune deficiencies and to fight infections.
Erythropoietin	Stimulates erythrocyte production; used to treat anemia in patients with kidney disease.
Growth factors	Stimulate differentiation and growth of various cell types; used to promote wound healing.
Human growth hormone	Used to treat dwarfism.
Human insulin	Used to treat diabetes.
Interferons	Interfere with viral reproduction; used to treat some cancers.
Interleukins	Activate and stimulate different classes of leukocytes; possible uses in treating wounds, HIV infection, cancer, and immune deficiencies.
Monoclonal antibodies	Extraordinary binding specificity is used in: diagnostic tests; targeted transport (of drugs, toxins, or radioactive compounds to tumors as a cancer therapy); many other applications.
Superoxide dismutase	Prevents tissue damage from reactive oxygen species when tissues briefly deprived of O <sub>2</sub> during surgery suddenly have blood flow restored.
Vaccines	Proteins derived from viral coats are as effective in “priming” an immune system as the killed virus more traditionally used for vaccines, but are safer; first developed was the vaccine for hepatitis B.

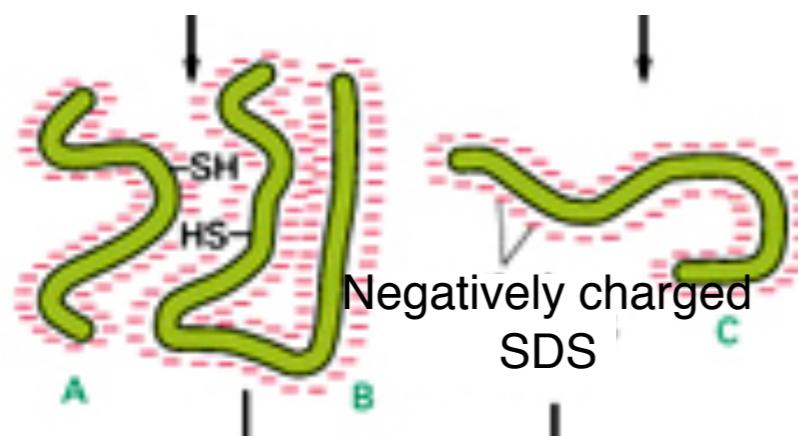
Protein with two subunits, A and B joined by a disulfide bridge



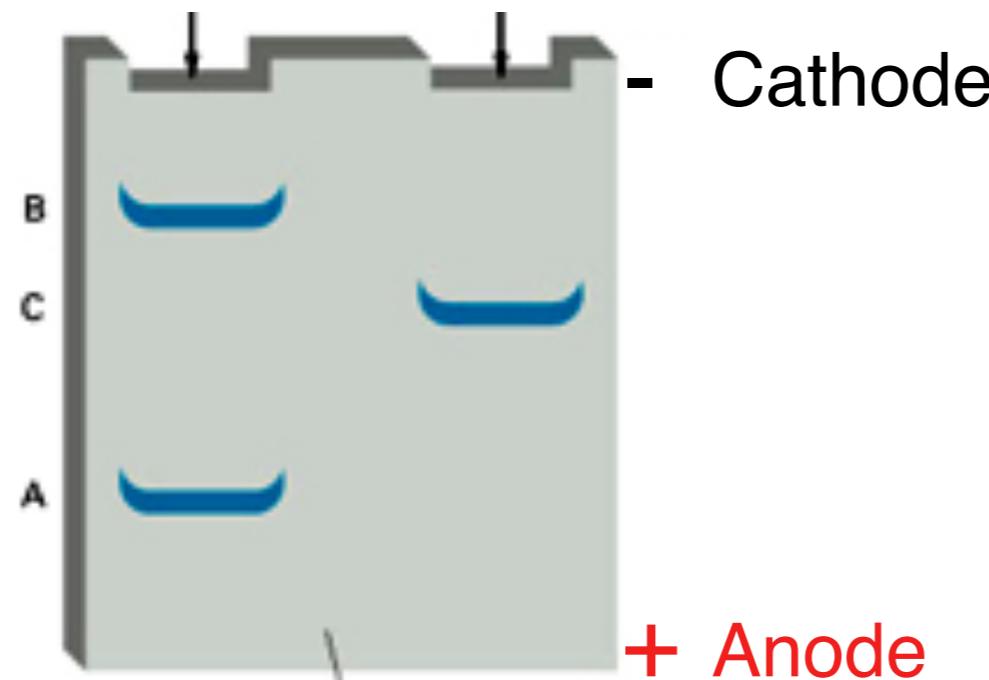
Single subunit protein



Heated with SDS and reducing agent



Polyacrylamide gel electrophoresis

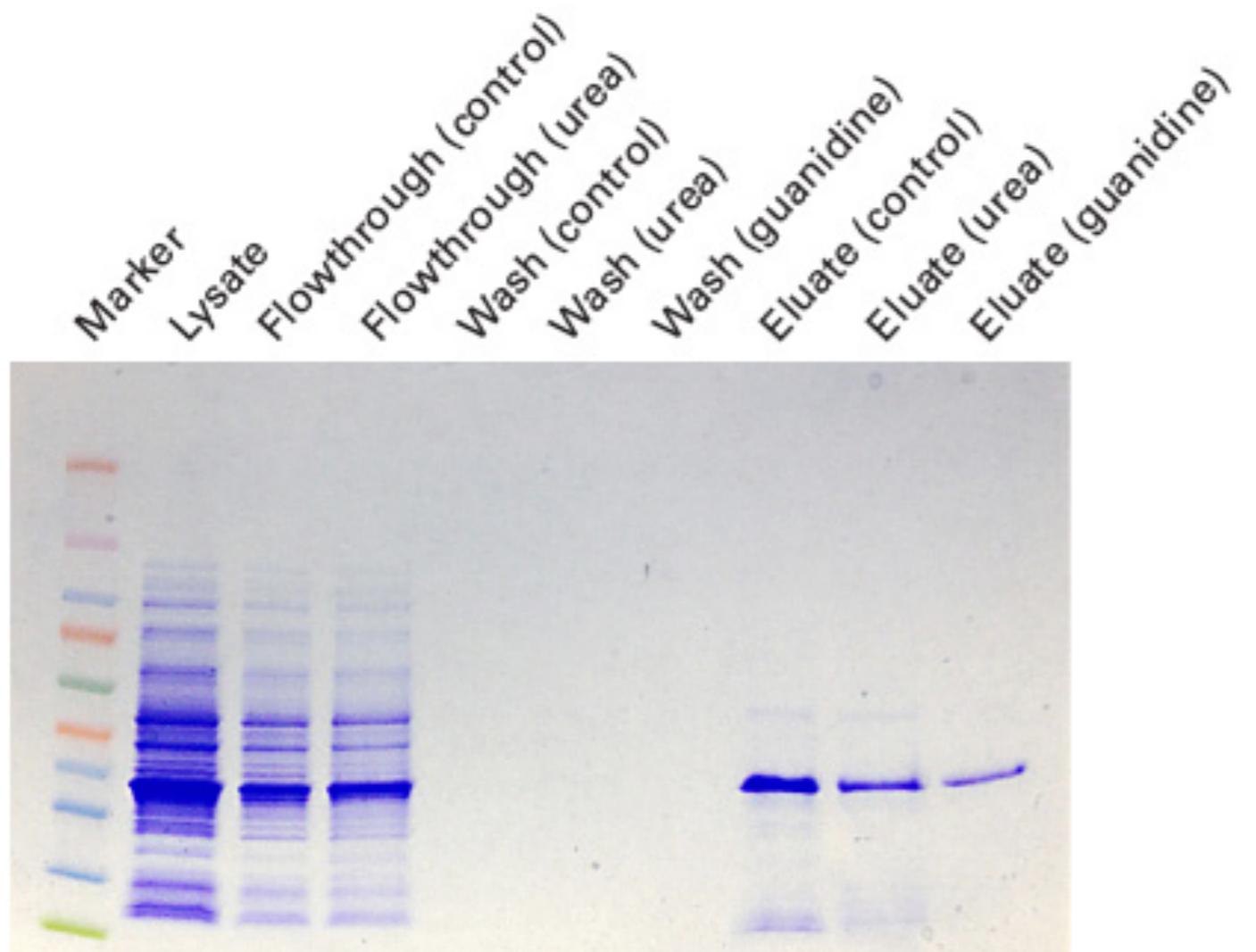


Polyacrylamide gel (stained to visualize proteins)

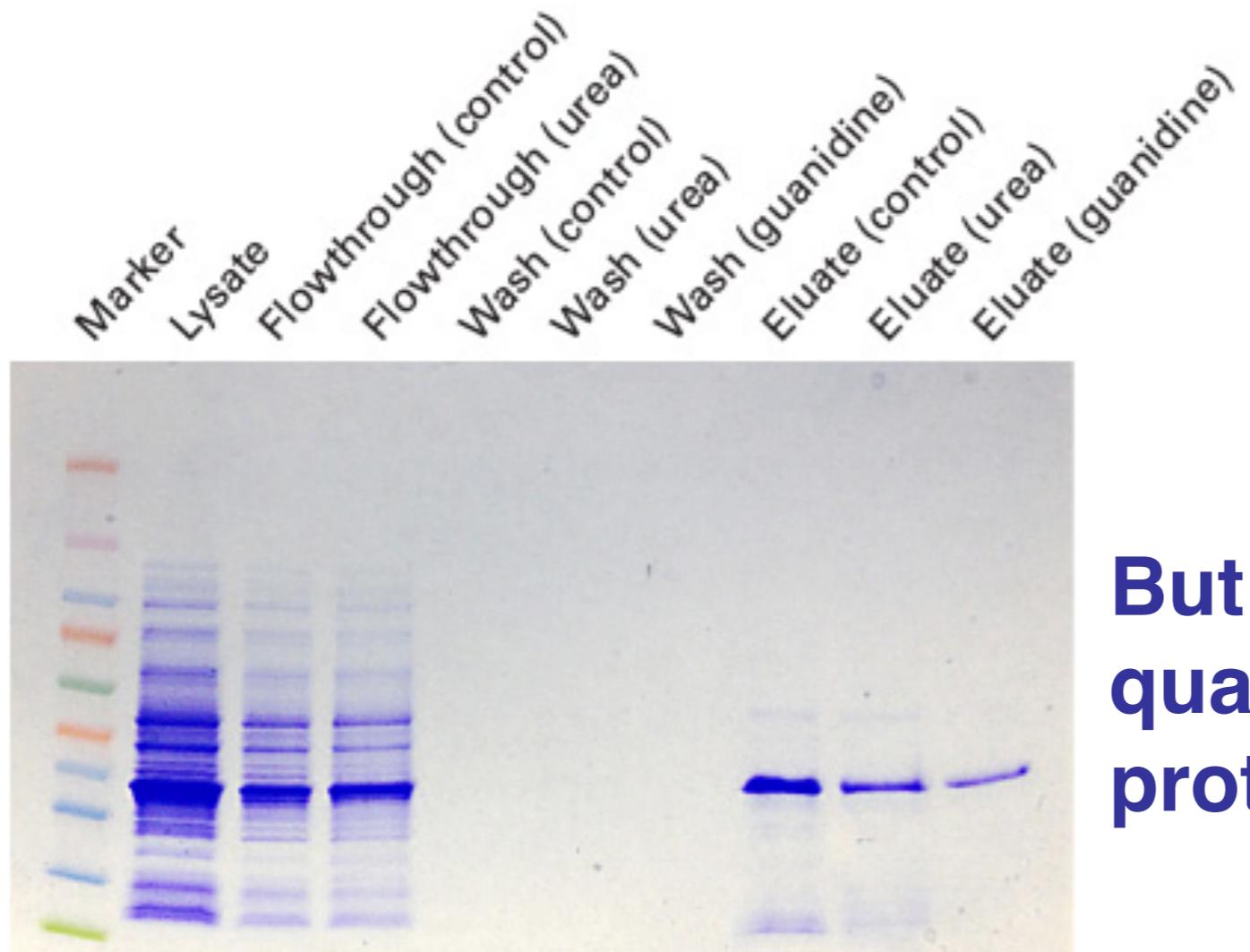
# SDS-PAGE separates proteins on the basis of size

Polypeptide chains form a complex with negatively charged molecules of the detergent sodium dodecyl sulfate (SDS), and migrate as a negatively charged SDS-complex through a polyacrylamide gel. Proteins migrate at a rate that reflects their molecular weight; smaller proteins migrate faster through the gel than larger proteins.

# Verification of purified protein

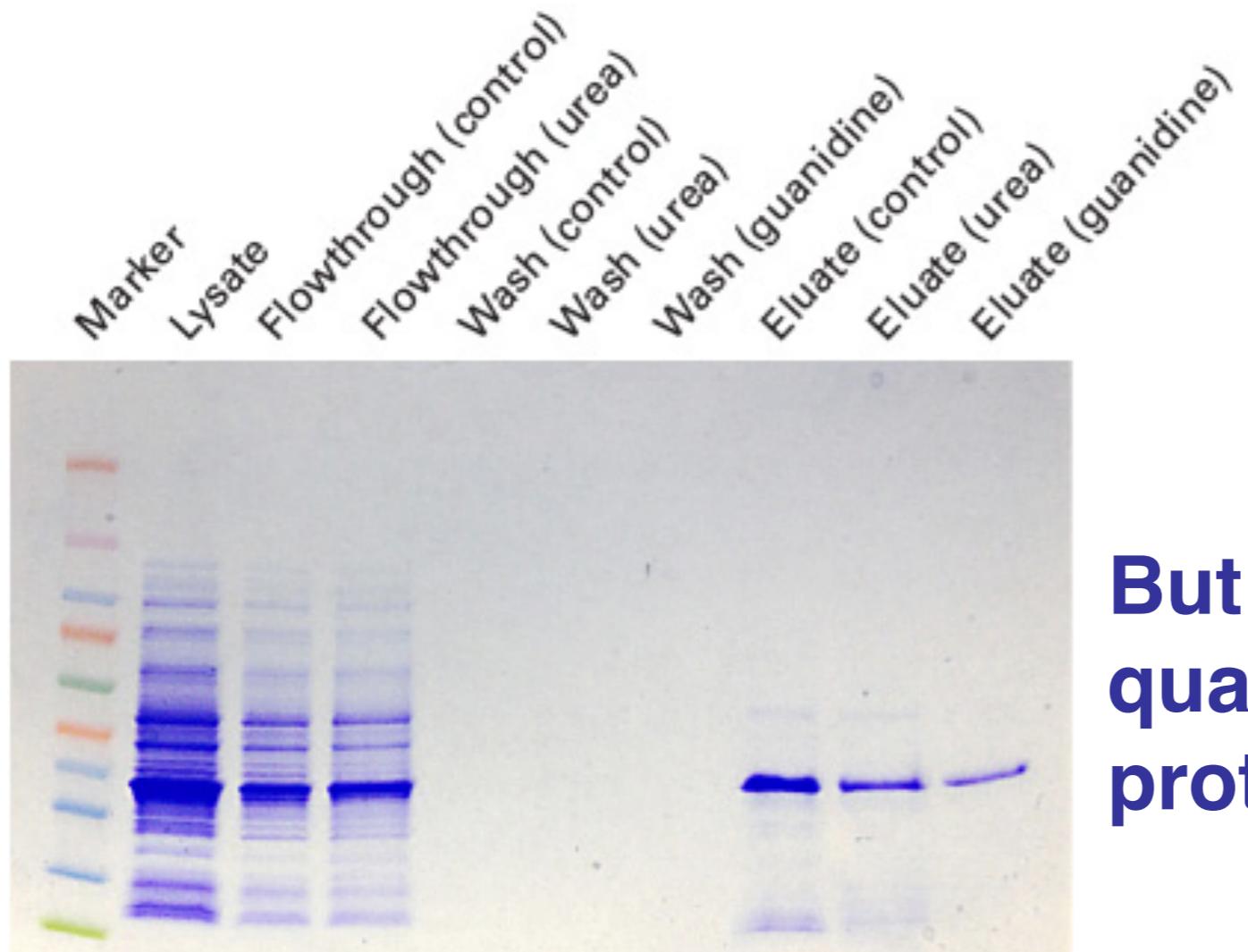


# Verification of purified protein



**But what if we just wanted to quantify the abundance of one protein within the lysate?**

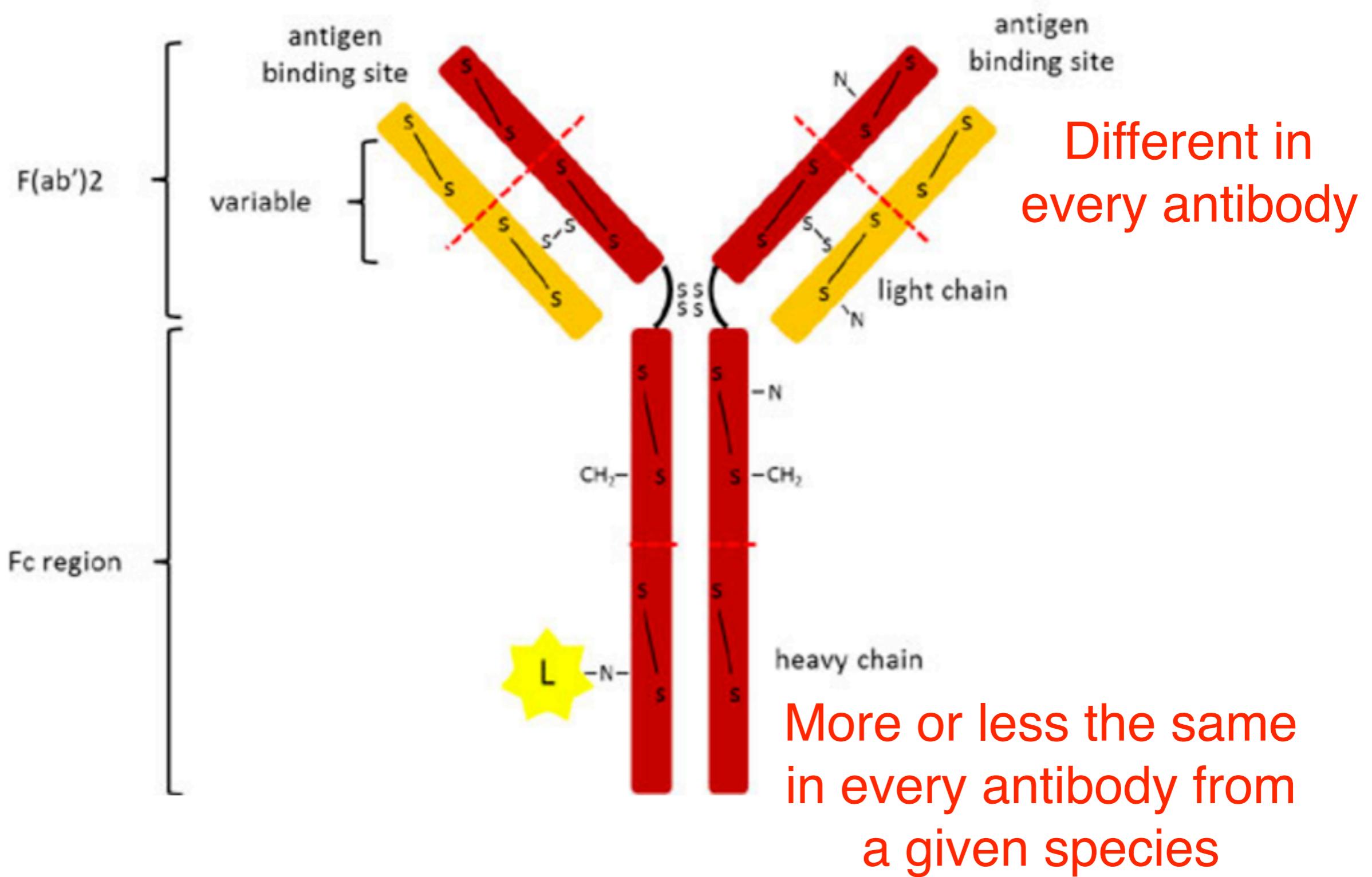
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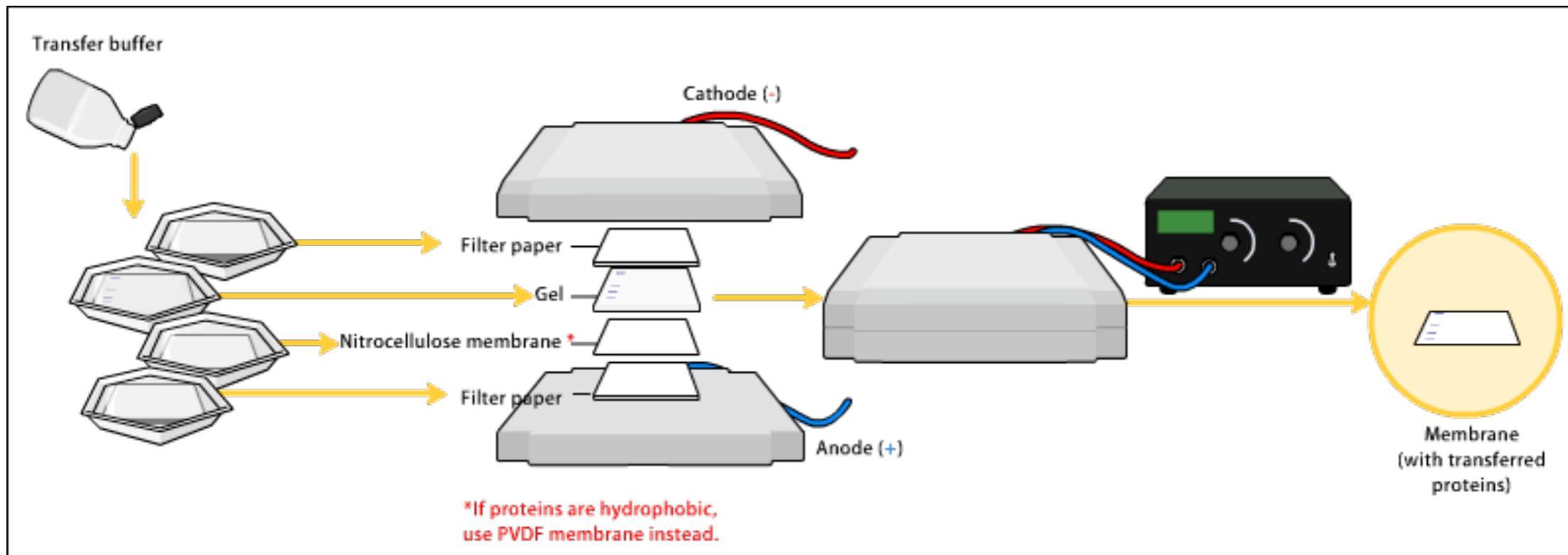
**Is a patient currently infected with...**  
**HIV?**  
**Lyme disease?**  
**Mad cow disease?**

# Antibodies recognize specific proteins



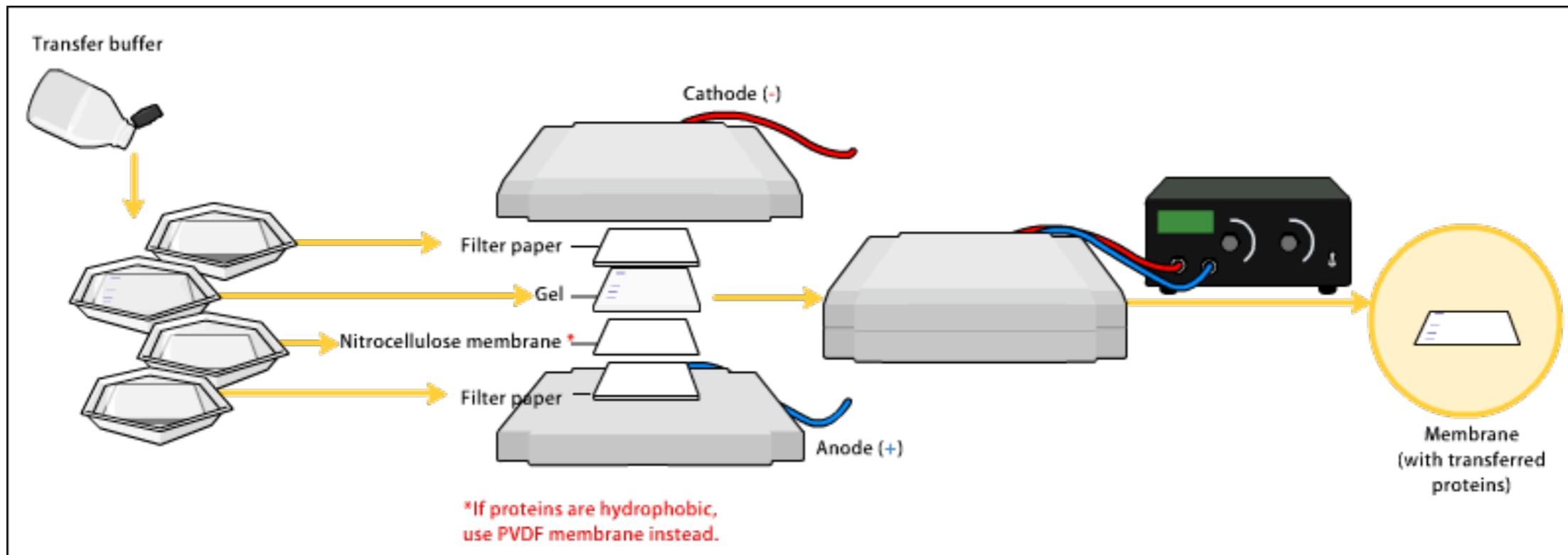
# Western blotting for protein quantification

Antibodies can't really get into a gel to bind proteins, so we transfer protein from a gel to a membrane



# Western blotting for protein quantification

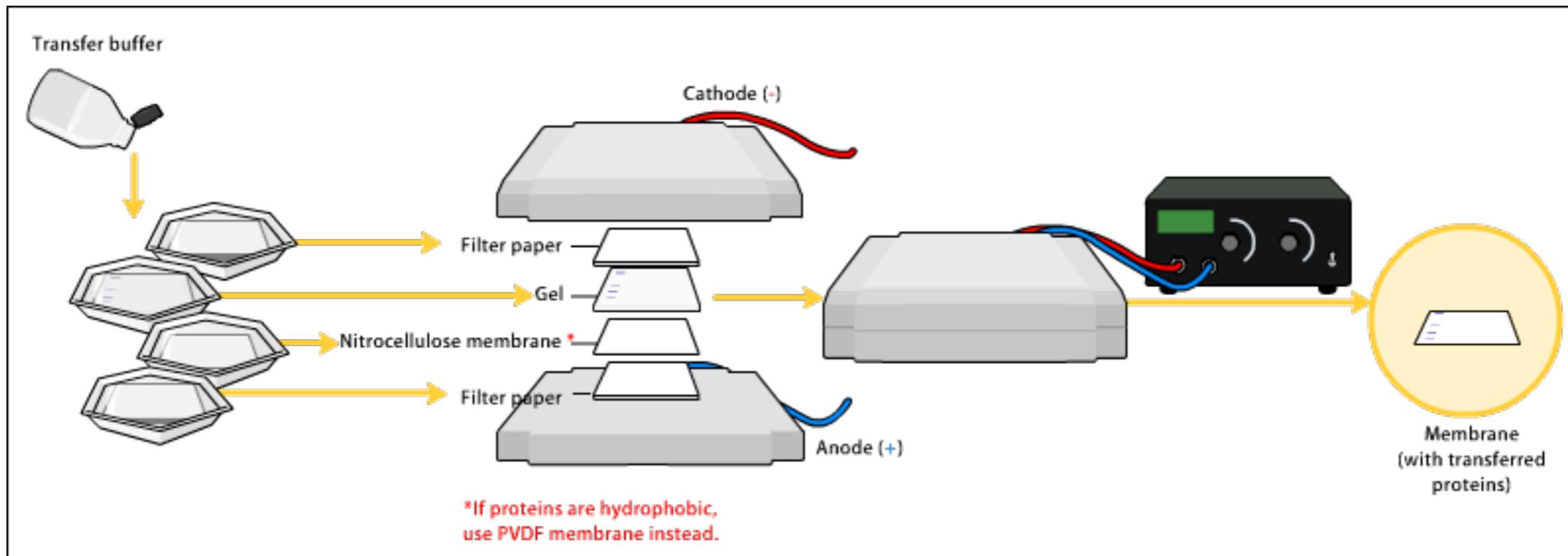
Antibodies can't really get into a gel to bind proteins, so we transfer protein from a gel to a membrane



But how to detect  
where the antibodies  
are on the membrane?

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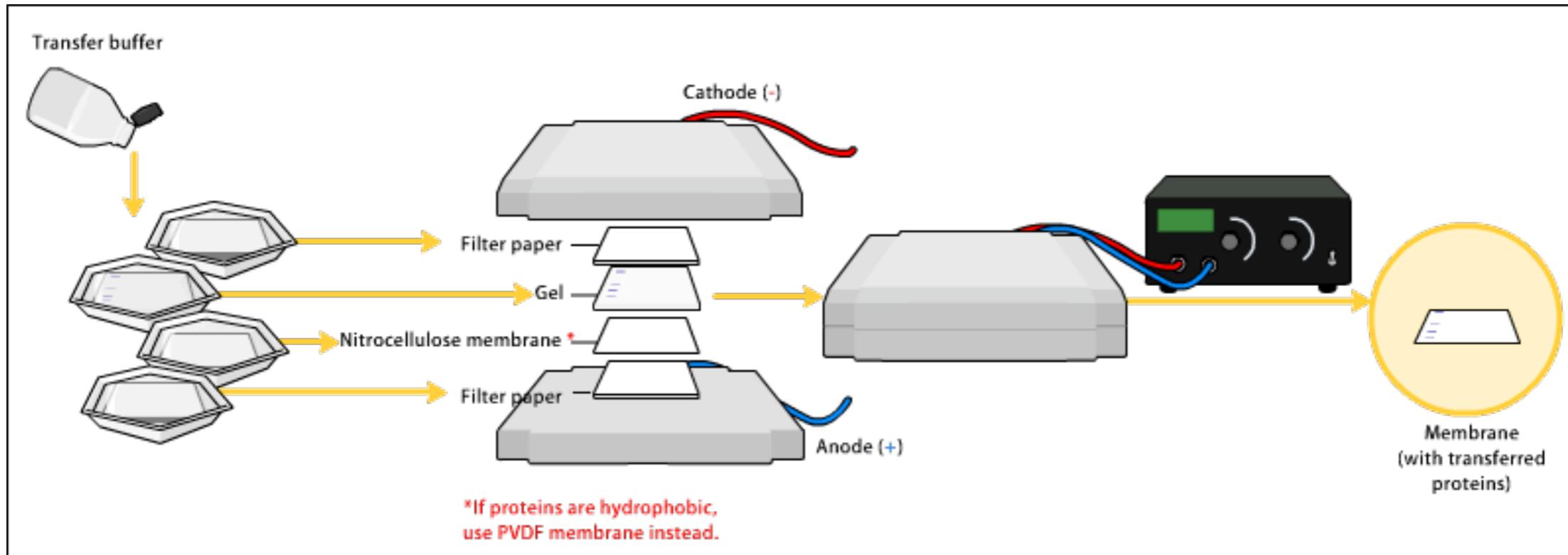


But how to detect  
where the antibodies  
are on the membrane?

Use another antibody!

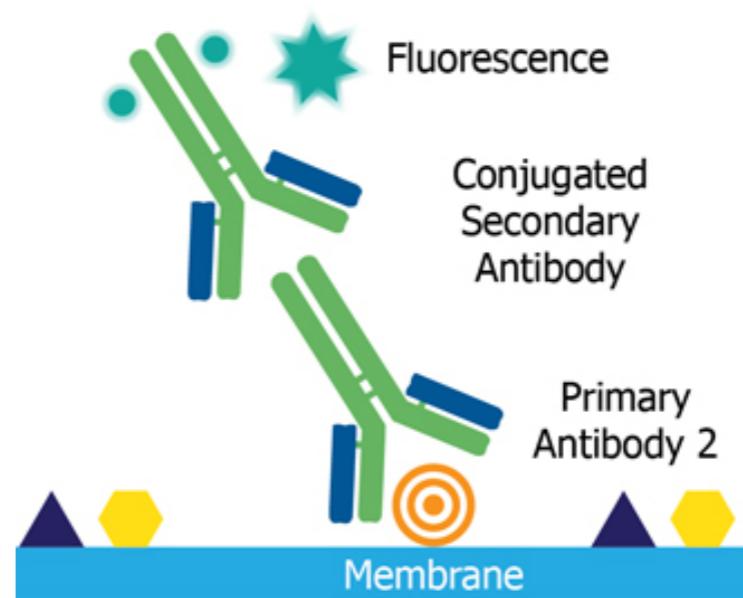
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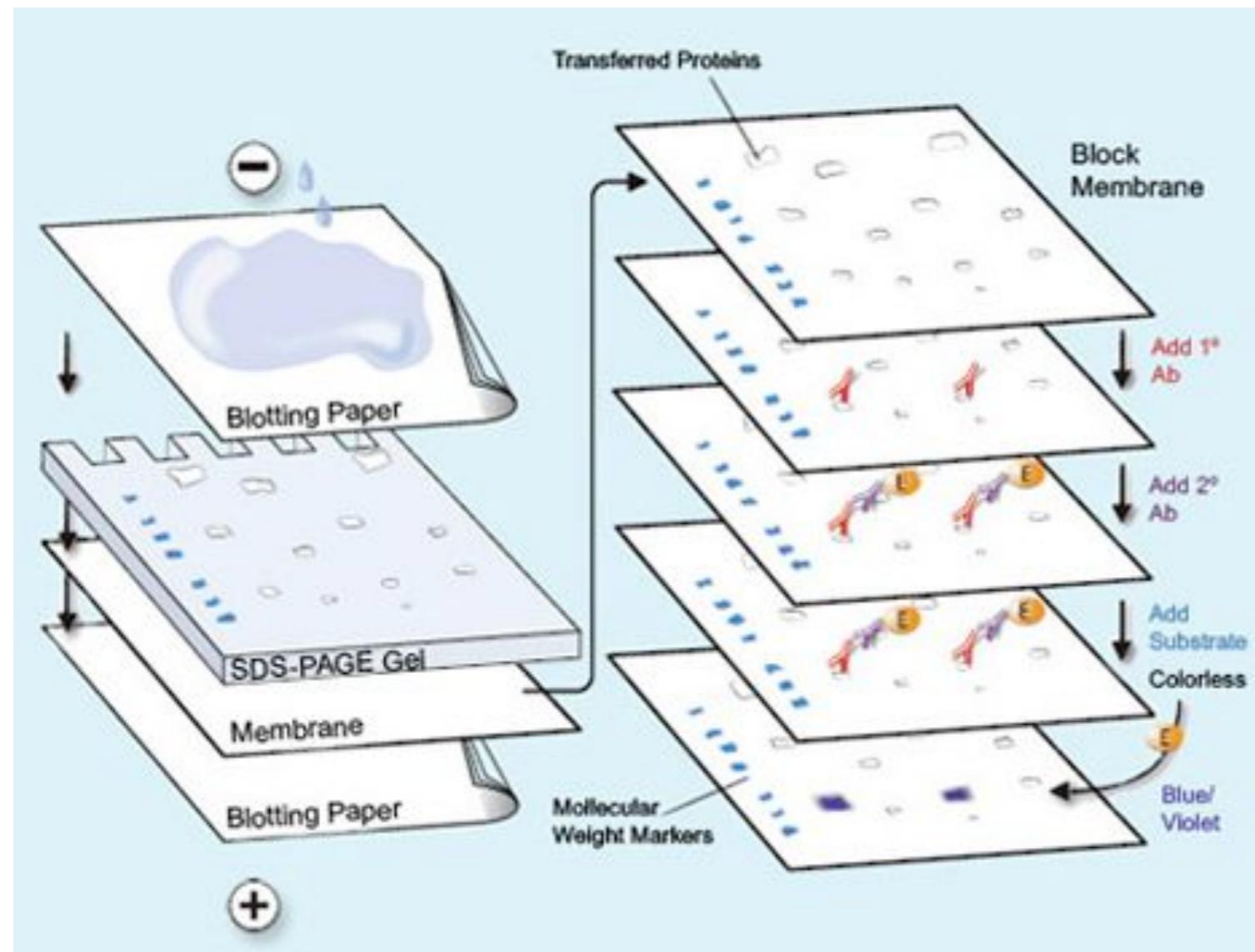


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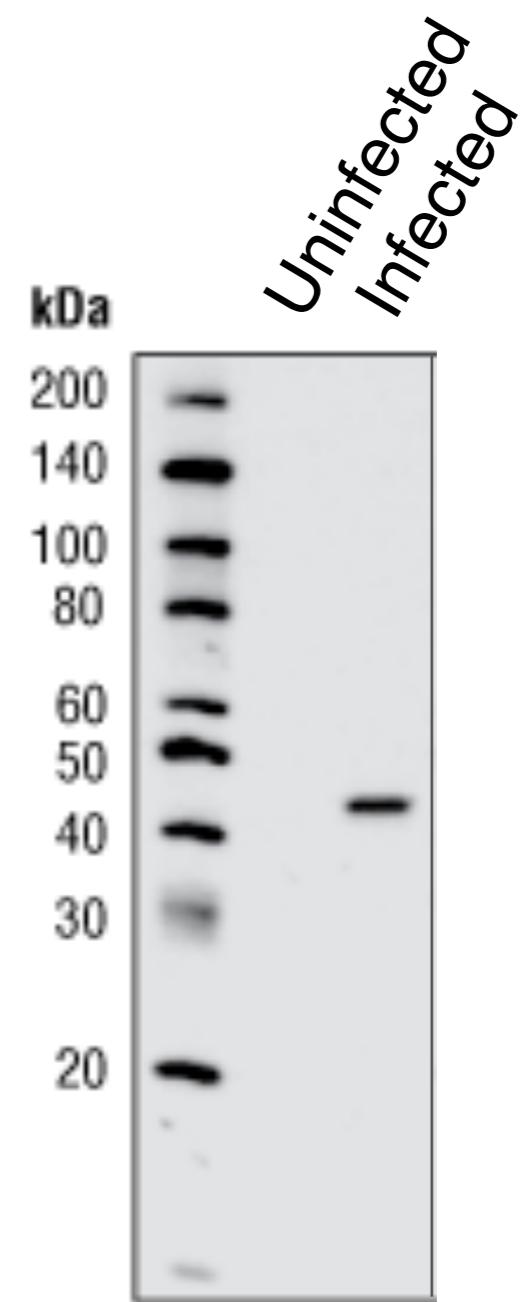
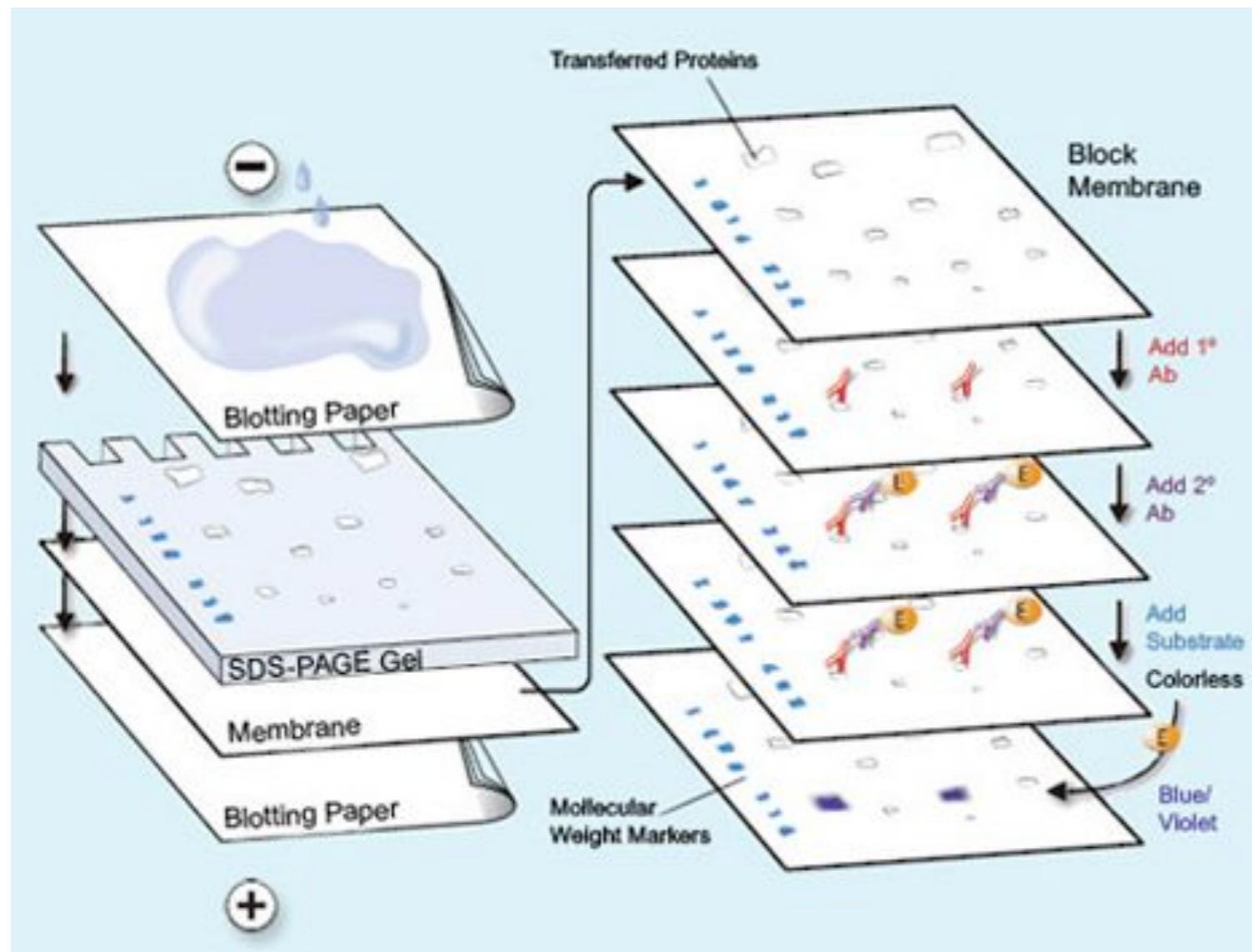
Use another antibody!



# Western blotting for protein quantification

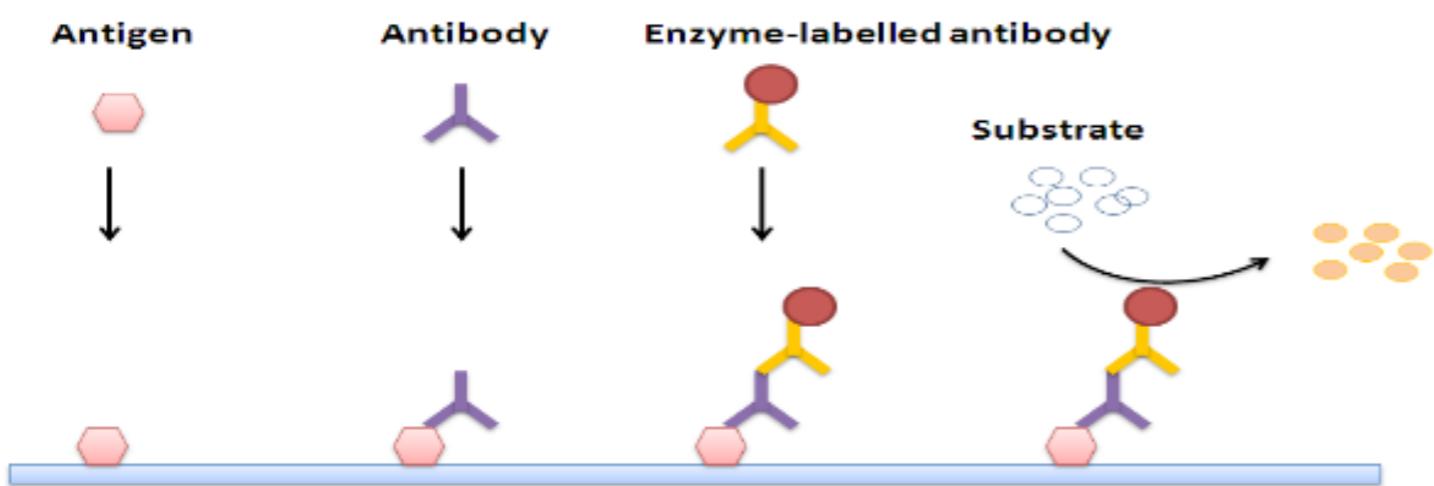


# Western blotting for protein quantification



# OK so Western blots tell us about the proteins currently in a sample, how could we monitor past exposure to an antigen?

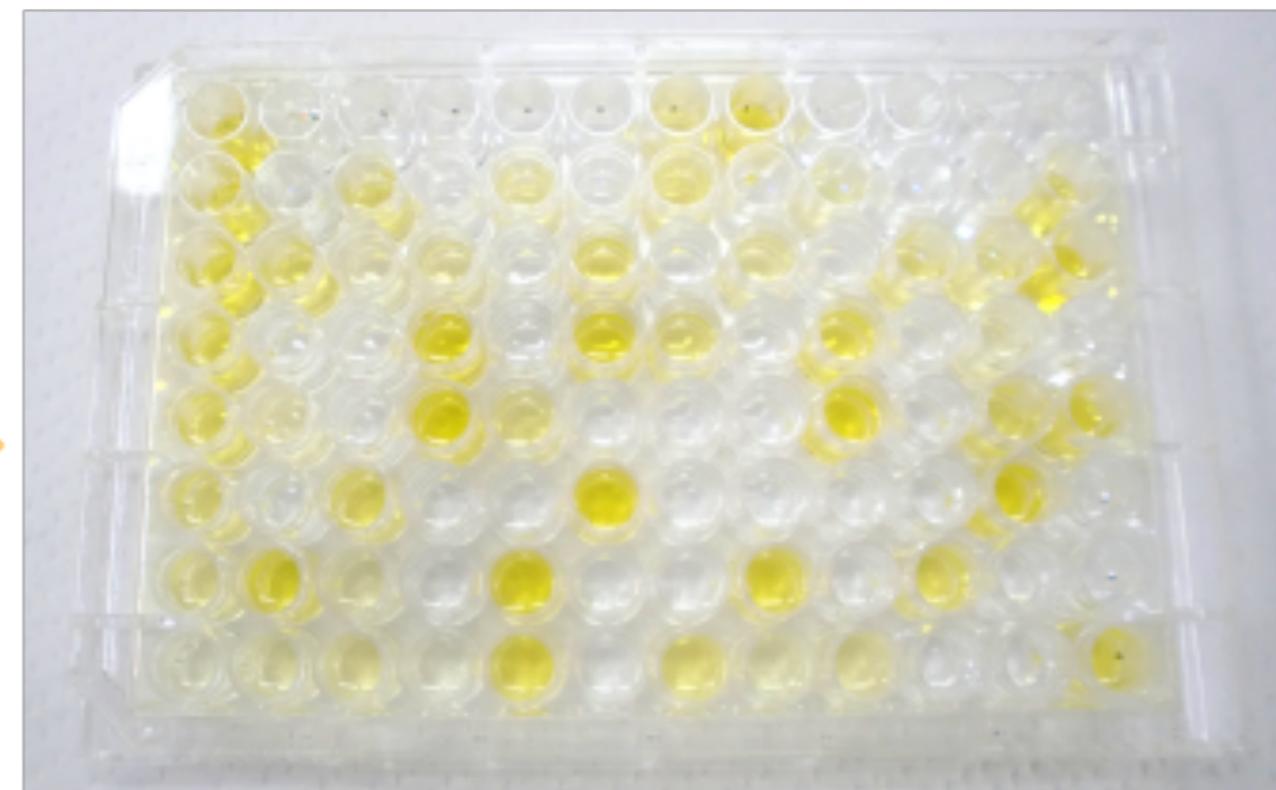
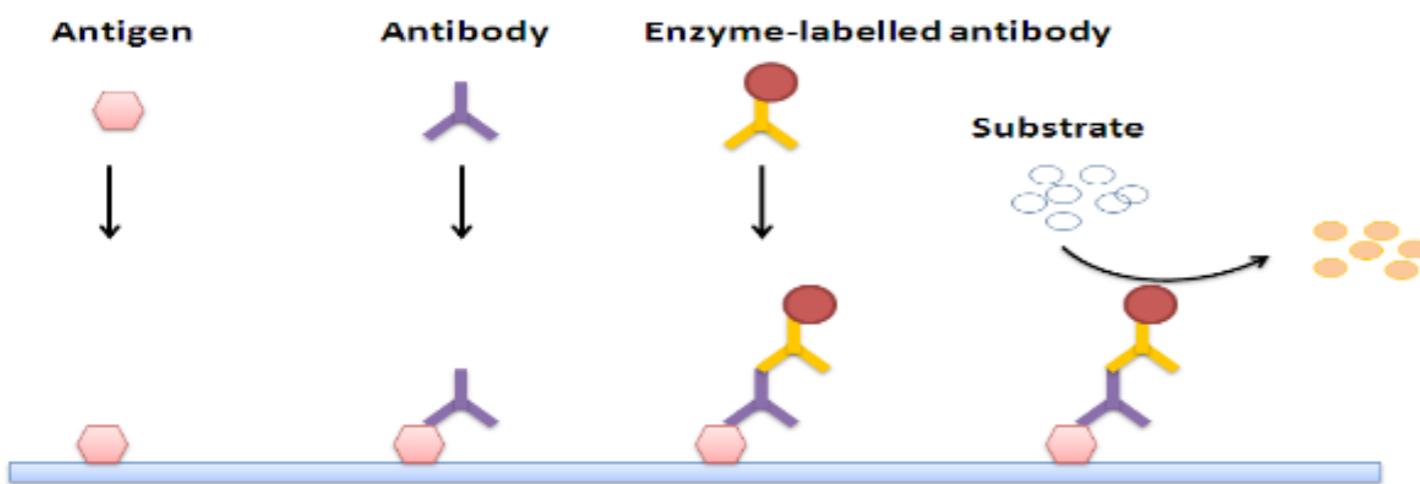
Detect the presence of antibodies in serum!



# OK so Western blots tell us about the proteins currently in a sample, how could we monitor past exposure to an antigen?

Detect the presence of antibodies in serum!

ELISA = Enzyme-linked immunosorbent assay



The yellow color indicates that the target protein is present.  
The higher degree of the color, the higher concentration  
of the target protein.

# MUDDIEST POINT

# MUDDIEST POINT

- A. Repeat expansion PCR
- B. DNA Fingerprinting
- C. Restriction fragment length polymorphism
- D. Sanger Sequencing
- E. Real time PCR
- F. Microarrays
- G. Recombinant protein production
- H. Antibody-based protein quantification